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Titel der Dissertation:

Salivary Aldosterone, Central and Peripheral Mineralocorticoid Receptor Function and Their Impact on the Course of Depression

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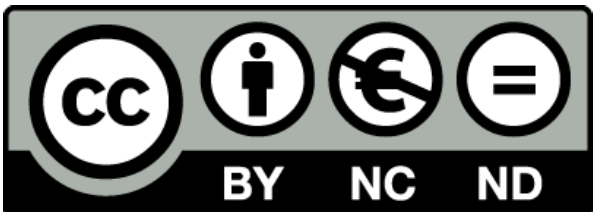

Meiner Mutter Theresia,
meinem Vater Peter
und meiner Frau Amelie

Abstract:

Background: Aldosterone and mineralocorticoid receptor (MR) function appear to play a role in depression. Central and peripheral biomarker parameters of MR function at baseline, their early (change within two weeks) and late (change within six weeks) plasticity were examined with regard to their relationship to clinical treatment outcome after six weeks in patients with acute major depression.

Methods: Twenty-four patients with major depression were examined three times during six weeks. Aldosterone and cortisol salvia samples were taken at 7:00 am before patients got out of bed. Easy to use e-devices were used to measure markers of central MR function, i.e. slow wave sleep (SWS) and heart rate variability (HRV). A newly developed scale determined salt taste intensity (STI) and salt pleasantness (SP) of a 0.9% salt solution. In addition, systolic blood pressure (SBP) and plasma electrolytes (Mg^{2+}, Na^{+}, K^{+}) were determined as markers of peripheral MR activity. The relationship between the levels of these biomarkers at baseline, their early and late plasticity and the relative change in clinical outcome parameters (Hamilton Depression Rating Scale with 6 and 21 items, QIDS-SR-16 and BDI) after six weeks of treatment was investigated.

Results: By trend a higher baseline aldosterone to cortisol ratio (aldo/cort) ($p < 0.1$) and lower baseline SBP ($p < 0.05$) predicted poor outcome, independent of gender. Only in male patients lower baseline SP, lower SWS and higher HRV predicted beneficial outcome ($p < 0.05$). Likewise, in male patients low baseline Na^{+} appeared to be by trend predictive for a poor outcome ($p = 0.050$).

Independent of gender an early cortisol reduction ($p < 0.05$) did predict clinical improvement, whereas by trend an early SWS increase was associated with better outcome ($p < 0.1$). Only in female patients an early Na^{+}/K^{+} ratio increase appeared to be related to a better outcome ($p < 0.05$).

By trend only in male patients a late HRV reduction was associated with beneficial outcome ($p < 0.1$).

Conclusion: Correlates of higher baseline central MR activation were associated with poorer clinical improvement, particularly in men. This contrasted with a lower sensitivity of peripheral MR function at baseline in more refractory patients. As one potential mechanism to consider, Na^{+} loss on the basis of dysfunctional peripheral

MR function and additional environmental factors may trigger increased aldosterone secretion and consequently lead to a less favorable outcome.

Results for the parameter plasticity were heterogeneous. In the course of depression markers of increasing central as well as peripheral MR activation were found to be related to a favorable outcome. These makers were: decreasing cortisol (independent of aldosterone), increasing SWS, decreasing HRV and increasing Na^+ plasma concentrations. Of note gender differences may exist in terms of MR function.

This study showcases the usefulness of biological markers, which can be obtained at bedside, to achieve individualized medicine in therapy refractory depression.

Der Einfluss von Speichelaldosteron sowie der zentralen und peripheren Mineralokortikoidrezeptor-Funktion auf den Verlauf einer Depression

Deutsche Zusammenfassung:

Hintergrund: Aldosteron und der funktionelle Zustand des Mineralokortikoidrezeptors (MR) scheinen eine Rolle bei der Depression zu spielen. Bei Patienten mit einer akuten depressiven Episode wurden zentrale und periphere Parameter der MR-Funktion, einerseits zu Beginn der Studie (Baseline), andererseits deren frühe (Veränderung innerhalb von zwei Wochen) und späte (Veränderung innerhalb von sechs Wochen) Plastizität, in Bezug auf das klinische Behandlungsergebnis nach sechs Wochen untersucht.

Methoden: Vierundzwanzig Patienten mit einer unipolaren Depression wurden dreimal innerhalb von sechs Wochen untersucht. Es wurden ihnen jeweils um 7 Uhr morgens vor dem Aufstehen Speichelproben zur Messung von Aldosteron und Kortisol entnommen. Um Marker der zentralen MR-Funktion, wie den Tiefschlaf (SWS) und die Herzfrequenzvariabilität (HRV), zu messen, wurden leicht zu handhabende elektronische Geräte benutzt. Nach einem Geschmackstest mit einer 0,9% Salzlösung legten die Patienten auf einer für diese Studie entwickelten Visuellen Analogskala die subjektive Salzgeschmacksintensität (STI) und Salzpräferenz (SP) fest. Zusätzlich wurden der systolische Blutdruck (SBP) und bestimmte Plasmaelektrolyte (Mg^{2+}, Na^{+}, K^{+}) als Marker für die periphere MR-Aktivität bestimmt. Es wurde der Zusammenhang zwischen der Höhe dieser Biomarker zu Beginn der Studie sowie deren frühe/späte Plastizität und der proportionalen Veränderung der Depressionsschwere (Hamilton Depressionsskala mit 6 und 21 Fragen, QIDS-SR-16 und BDI) nach sechs Wochen ermittelt.

Ergebnisse: Im Trend sagte ein höheres Verhältnis von Aldosteron zu Kortisol (aldo/cort) ($p < 0,1$) und ein niedriger SBP ($p < 0,05$) bei Baseline eine schlechtere proportionale Depressionssymptomatik nach sechs Wochen voraus. Dieses Ergebnis war unabhängig vom Geschlecht. Eine niedrige SP, ein geringer SWS und eine hohe HRV bei Baseline zeigten nur bei Männern eine positive Auswirkung auf den relativen Depressionszustand ($p < 0,05$). Ebenso erwies sich bei Männern ein

niedriges Baseline-Na^+ im Trend prädiktiv für einen schlechteren relativen Depressionszustand ($p = 0,050$).

Es stellte sich heraus, dass eine frühe Kortisolreduktion ($p < 0,05$) unabhängig vom Geschlecht prädiktiv für eine klinische Verbesserung des relativen Depressionszustandes ist. Ein früher SWS-Anstieg war im Trend mit einer Verbesserung des relativen Depressionszustandes assoziiert ($p < 0,1$). Bei Frauen wirkte sich ein früher Na^+/K^+-Anstieg positiv aus ($p < 0,05$).

Im Trend war bei Männern eine späte HRV-Reduktion mit einer klinischen Verbesserung des relativen Depressionszustandes assoziiert ($p < 0,1$).

Schlussfolgerung: Baselinekorrelate einer hohen zentralen MR-Aktivierung waren assoziiert mit einer geringeren klinischen Verbesserung nach sechs Wochen. Dies war vor allem bei Männern nachweisbar. Im Gegensatz dazu zeigte sich bei therapierefraktären Patienten bei Baseline eine geringere MR-Sensitivität in der Peripherie. Eine mögliche Erklärung dafür stellt ein Na^+-Verlust auf der Grundlage einer von Umweltfaktoren begleiteten dysfunktionalen peripheren MR-Funktion dar. Diese periphere MR-Dysfunktion bedingt nachfolgend eine erhöhte Aldosteronsekretion und ein schlechteres Therapieansprechen.

Die Ergebnisse für die Parameterplastizität im Verlauf der Depression waren heterogen. Hierbei lag bei Patienten mit einer Verbesserung der Depression sowohl eine steigende zentrale als auch periphere MR-Aktivierung vor. Dies zeigte sich durch eine Kortisolreduktion (unabhängig von Aldosteron), einen SWS-Anstieg, eine HRV-Reduktion und einen Anstieg der Plasmanatriumkonzentration. Dabei spielt das Geschlecht hinsichtlich der MR-Funktion eine Rolle.

Diese Studie hebt die Bedeutung von Biomarkern, die am Patientenbett erhoben werden können, hervor. Auf dem Weg zu einer individualisierten Medizin bei der Behandlung therapierefraktärer Depressionen stellt dies einen wichtigen Schritt dar.

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1. Introduction

1.1. Therapy response in depression

Major depressive disorder is a frequent, individually impairing and economically highly expensive condition. One in four women and one in eight men experience such depression throughout their lifetime. In total it has a lifetime prevalence of worldwide 16% (Kessler et al., 2003) out of which every fifth depression takes a chronic course (Kennedy et al., 2003). The 2008 World Health Organization report 'The global burden of disease' lists unipolar depression in middle and high-income countries as the number one disease for the leading causes of burden of disease with up to 29 million disability-adjusted life years (DALY) (World Health Organization, 2008). *'One main limitation of current treatments is the lack of predictive markers of treatment response, which would highlight the need for alternative treatment strategies. This is highly relevant as existing pharmacological treatment options are not satisfactory (Fava et al., 2003; Trivedi et al., 2006),'* (Büttner et al. 2015: 24).

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study examined over 2800 patients suffering from depression in an open label sequential design and observed only poor remission rates. Remission was particularly bad in patients additionally exhibiting somatic symptomatology (Silverstein and Patel, 2011), atypical features (Stewart et al., 2010), anxiety, and metabolic syndrome (Richter et al., 2010). The poor results of remission and response to antidepressant medication could point to heterogeneity of pathophysiological causes in depression (Büttner et al., 2015). This is especially important in patients with atypical features of depression, which concerned about 19% of the STAR*D population. These patients were less likely to remit with common antidepressant medication (Stewart et al., 2010). Even poorer response rates can be seen in data of antidepressant medication studies analyzing the most widely prescribed antidepressants. This database is derived from placebo controlled clinical trials and contains all data (published and non-published), which were submitted to the United States Food and Drug Administration. The data indicates that only 18 – 32% of the drug effect size can be attributed to the pharmacological effect of the medication (Kirsch et al., 2002; E. H. Turner et al., 2008).

1.2. Depression has a complex impact on the body

According to the 10th revision of the International Classification of Diseases and Related Health Problems (ICD-10) a major depressive episode is characterized by at least two out of three main symptoms like a lowered, depressive mood, loss of interests, and/or the lack of drive. These main symptoms are accompanied by at least two additional symptoms, such as a decreased concentration and attention span, decreased levels of self-esteem and self-confidence, feelings of guilt and worthlessness, negative and pessimistic perspective of the future, suicidal thoughts or behavior, sleep disorder and/or decreased appetite. To diagnose a major depressive episode according to ICD-10, these symptoms have to persist for at least two weeks.

The heterogeneity in unipolar depression is based on psychological differences as well as differences in somatic functions. The origin and course of depression are influenced by somatic factors, such as an *'unhealthy lifestyle (Luppino et al., 2010), metabolic factors (Kahl et al., 2012), […] inflammation (Dowlati et al., 2010; Howren et al., 2009) […], status of the autonomic nervous system (Guinjoan et al., 1995; Lehofer et al., 1997), and the HPA [(hypothalamus-pituitary-adrenal)] axis as being part of the stress hormone system (Vreeburg et al., 2009). This interaction between somatic and mental conditions is also reflected in the observation that many somatic diseases are associated with depression: depression seems to be an independent risk factor for coronary artery disease (Nicholson et al., 2006; Penninx et al., 2001), hypertension (Meng et al., 2012) [(Dhar and Barton, 2016)] and cerebral insult (Dong et al., 2012). Patients suffering from unipolar depression may have an up to 2.4 higher prevalence for metabolic syndrome (Kahl et al., 2012), but reported results are heterogeneous (Heiskanen et al., 2006; Herva et al., 2006). Conversely, major depressive disorder has been associated with an increased incidence of type 2 diabetes mellitus and cardiovascular disorders (Laaksonen et al., 2002; Lakka et al., 2002; Mezuk et al., 2008).'* (Büttner et al. 2015: 25).

Greater relative body weight increases non-response to antidepressant treatment, especially to fluoxetine (Papakostas et al., 2005). Several of these cardiovascular risk factors are associated with a defined subgroup of depression, i.e. atypical depression as listed in the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) and mentioned under F32.8 'Other depressive episodes' in the 2014 German modification of the ICD-10.

Atypical depression is defined by specific clinical characteristics, i.e. hyperphagia, hypersomnia, irritability (rejection sensitivity) and somatoform complains (leaden paralysis). The metabolic syndrome, inflammatory markers and body mass index (BMI) seem to be associated with atypical depression (Lamers et al., 2012b). *'Other moderators of treatment response as well as neuroendocrine characteristics are related to early life trauma (Juruena, 2014; Juruena et al., 2015; Werne Baes et al., 2013).*

Whereas the role of the […] HPA axis in depression has been widely reported (Holsboer, 1995), the focus is often related to changes in cortisol concentration and the glucocorticoid receptor (GR).' (Büttner et al. 2015: 25).

Nevertheless, it is very important to remember that aldosterone secretion is stimulated by adrenocorticotropic hormone (ACTH) which stimulates cortisol as well as aldosterone secretion at the adrenal glands.

'More recently the […] [mineralocorticoid receptor] MR received wider attention see (Heegde et al., 2015) for a recent review. […] [Some years ago], aldosterone, the physiological ligand for the MR, has been recognized as a potential marker of depression (Murck et al., 2003), in particular in patients, who show a co-occurrence of depression and arterial hypertension (Hafner et al., 2013). […] [It was] pointed out earlier that one potential biological mechanism that shows an overlap between metabolic disturbances, inflammatory changes and depression could be an increased activity of the renin-angiotensin-aldosterone system [(RAAS)], including an increase in aldosterone release (Murck et al., 2012),' (Büttner et al. 2015: 25).

Data from experimental animal research points to the early occurrence of aldosterone release under stressful conditions (Franklin et al., 2012) and further points to a direct effect of aldosterone administration to induce depression- and anxiety-like behavior (Hlavacova et al., 2012). Aldosterone is a classic part of the stress hormone axis (Selye, 1955), even though it is widely neglected as such (Büttner et al., 2015). The early discovery of the proinflammatory action of aldosterone by Selye has since then been widely confirmed (Felder, 2010; Johnson and Grippo, 2006). *'A potential contributory role of aldosterone in a subset of depression is implied by the increased rate of anxiety, depression and somatization (Sonino et al., 2011) as well as metabolic syndrome in patients with hyperaldosteronism (A. D. de Kloet et al., 2010).'* (Büttner et al. 2015: 25).

1.3. Aldosterone sensitive sites in the central nervous system

To respond to physical stress, the HPA axis is one of the main regulatory systems and can be linked to the limbic system and other mood regulating neuronal networks (Figure 17). Corticotropin-releasing hormone (CRH) is produced in the paraventricular nucleus (PVN) of the hypothalamus to release ACTH. ACTH is released by the anterior pituitary gland and circulates through the blood to stimulate the secretion of cortisol and aldosterone at the adrenal glands. Aldosterone is mainly produced by the zona glomerulosa and cortisol by the zona fasciculate of the adrenal glands (Figure 1). Generally, cortisol is considered to be the 'classical stress hormone'. Both, aldosterone and cortisol are vital for situations of acute stress as they prepare the body for a 'fight-or-flight' response and fade away physiologically after the stress trigger disappears.

Figure 1: Structures of the steroid hormones aldosterone and cortisol

According to the International Union of Pure and Applied Chemistry aldosterone is also known as 11ß, 21-Hihydroxy-3,20-dioxopregn-4-en-18-al and cortisol as (11ß)-11,17,21-trihydrocypregn-4-ene-3,20-dione.

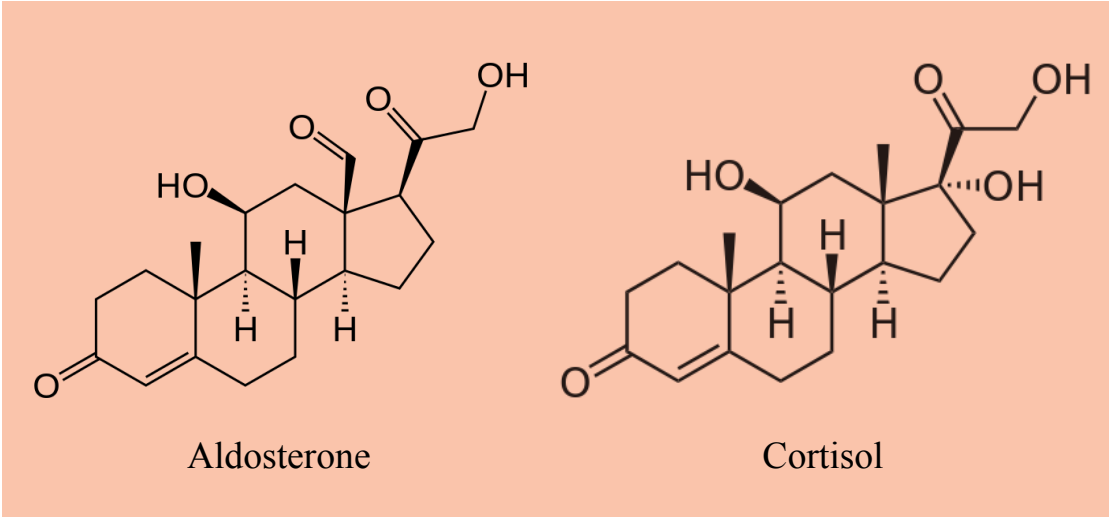


Image source: en.Wikipedia.org; http://en.wikipedia.org/wiki/Aldosterone#/media/File:Aldosterone-2D-skeletal.svg & http://en.wikipedia.org/wiki/Cortisol#/media/File:Cortisol2.svg

In this work the focus lies on aldosterone as it shares the regulation over ACTH in the HPA axis and is part of the RAAS where its synthesis is stimulated by angiotensin II. Other regulatory mechanisms that have an impact on aldosterone synthesis via the RAAS are the sympathetic nervous system (SNS), baroreceptors, plasma sodium

(Na^+) and potassium (K^+) concentrations (Francis et al., 2001; Guyenet, 2006; Schweda, 2015).

Aldosterone and cortisol unfold their activity over the MR and the GR. Steroids like aldosterone and cortisol enter the cell through passive diffusion, where they interact with the transcription factor MR. After this interaction the MR undergoes a nuclear translocation and binds specific hormone responsive elements at the level of the deoxyribonucleic acid (DNA), which leads to the transactivation of various target genes (Viengchareun et al., 2007). The MR and the GR are expressed in many different tissues and cell types of the body and have various functions. In some regions of the central nervous system (CNS) MR and GR are co-localized. The classical pathway of MR action is intracellular binding of aldosterone. Aldosterone is the primary physiological ligand of the MR and has a more stable connection to MR than cortisol (Lombes et al., 1994; Rogerson and Fuller, 2003). However, due to cortisol's 100 to 1000 fold higher concentration MR is less occupied by aldosterone in most tissues (E. P. Gomez-Sanchez and C. E. Gomez-Sanchez, 2012). Therefore, under basal conditions, for example in the hippocampus, cortisol is considered to primarily occupy the MR.

Due to different concentrations in the CNS the MR is most widely occupied by cortisol. In particular, the hippocampus has the highest density of MR in the brain (E. R. de Kloet et al., 1998). Besides the hippocampus this is also the case in cardiac myocytes, which are MR-expressing tissues and where the MR is mostly occupied by cortisol (Yang et al., 2011). However, a small number of areas exist in the CNS, which have the precondition for aldosterone to activate the MR. These areas express the enzyme 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD_2). This enzyme 'protects' the MR from the occupation of cortisol (Geerling and Loewy, 2009). Potential areas with 11ß-HSD_2 expressions are the PVN and the ventromedial nucleus of the hypothalamus, which regulate the release of ACTH and consecutively cortisol and aldosterone via the HPA axis, and the amygdala. Most relevant are the brainstem nucleus of the solitary tract (NTS) and potentially other brainstem areas like the locus coeruleus and the medial vestibular nucleus (E. P. Gomez-Sanchez and C. E. Gomez-Sanchez, 2012). These areas are part of a neuronal network of the neurobiology of emotions, feelings and depression (Damasio and Carvalho, 2013).

'The action of aldosterone in influencing central MR function is complex. Overall three different constellations of central MR function, depending on anatomical area and specific receptor type, are important.

1. For the selective activity of aldosterone, which acts classically at intracellular receptors, the co-expression of the MR and the enzyme […] [11ß-HSD$_2$] is required [for its direct impact on the DNA gene transcription]. This enzyme "protects" the MR from binding with cortisol. In the hippocampus, the anatomical area with the highest MR density, this is not the case. Therefore, at this site cortisol/corticosterone appears to be the primary ligand of the MR (E. R. de Kloet et al., 2000).

2. At the level of the hippocampus [and the amygdala] a membrane-bound, non-classical MR exists, which is not protected by […] [11ß-HSD$_2$], but nevertheless has some specificity for aldosterone: Its affinity for aldosterone is higher in comparison to cortisol/corticosterone (Karst et al., 2005).' (Büttner et al. 2015: 26).

This membrane-bound MR facilitates fast synaptic transmission. It is present on post-synaptic membrane densities of excitatory neurons and therefore directly modulates synaptic plasticity in the presence of steroid hormones (Prager et al., 2010). The membrane-bound MR is considered to be part of the rapid adaptive response to stress, as the genomic signaling via the genomic acting MR has a delayed response (Groeneweg et al., 2012).

'3. The most relevant anatomical areas in the current context are the specific areas in the brain, in which the MR and […] [11ß-HSD$_2$] are co-expressed and at which aldosterone can act specifically. These areas were originally thought to be relevant mainly in water and electrolyte regulation (E. R. de Kloet et al., 2000). More recently a closer characterization identified the […] NTS, the amygdala, the […] PVN of the hypothalamus as relevant targets (Geerling et al., 2006; Geerling and Loewy, 2009). Directly and through their connections these areas are involved in neuroendocrine regulation, salt appetite, sleep and, blood pressure regulation ([see] Murck et al. 2014 for review). From a syndromal perspective these regions are involved in anxiety and depression as well as emotions, and body perception. They are therefore potentially relevant for unexplained somatic complaints (somatization), which often co-occur with depression (Damasio and Carvalho, 2013).' (Büttner et al. 2015: 26).

The NTS plays a central part in the regulation of the vegetative nervous system and the release of stress hormones (Geerling and Loewy, 2009). Its connections to the

amygdala and the nucleus accumbens are associated with anxiety, motivational behavior and learning as part of a forebrain system implicated in reward notably for salt appetite and salt-seeking behavior of Na^+-depleted rats (Shekhtman et al., 2007; Voorhies and Bernstein, 2006). Other synaptic connections of the NTS are to the lateral parabrachial and prelocus coeruleus nuclei in the brainstem (Shekhtman et al., 2007).

Besides the NTS another important location of central aldosterone action is the PVN, which regulates the HPA axis over CRH secretion that releases downstream ACTH in the pituitary. It is sensitive to feedback mechanisms via ACTH, cortisol and aldosterone, but is also activated by angiotensin II (Saavedra et al., 2005) and therefore has an important influence on salt appetite, sympathetic drive and volume regulation (E. R. de Kloet et al., 2000) for example in congestive heart failure (Felder et al., 2001). Therefore, the PVN could be one potential link between the pathophysiological mechanism for depression and heart disease (Grippo and Johnson, 2002).

RAAS alterations in depression:

Evidence shows that aldosterone and the RAAS are involved in the pathogenesis of depression (Murck et al., 2012). Animal models and preliminary clinical studies point to an involvement of aldosterone in depression. In rats sub-chronic administration of aldosterone leads to anxiety and depression like behavior while at the same time inducing a change of gene transcription in the hippocampus that is associated with inflammation, glutamatergic activity, synaptic and neuronal remodeling pointing to a relationship between hyperaldosteronism and depressive behavior (Hlavacova et al., 2012). Several other animal models of depression show that high aldosterone levels appear before the occurrence of behavioral changes (Franklin et al., 2012). Injection of spironolactone, an mineralocorticoid receptor antagonist, leads to anxiolytic like effects in the elevated plus maze test (Korte et al., 1995).

In the plasma of patients suffering from major depression aldosterone is elevated (Emanuele et al., 2005; Murck et al., 2003). However, a clear cutoff defining elevated aldosterone levels has not yet been found. Depression-like symptoms have been found in patients with primary hyperaldosteronism (Künzel, 2012). The combination of depressive symptomatology and living alone was found to elevate the RAAS activity which then can consecutively cause somatic complications (Hafner et al., 2012).

Compared to controls, in suicide victims with a history of major depressive disorder an MR/GR ratio alteration in the hippocampus exists (Lopez et al., 1998). In these victims only MRs but not GRs are reduced. These gene expression changes are similar to those found in animals subjected to chronic unpredictable stress where it causes a decrease in the MR messenger ribonucleic acid (mRNA) levels in the hippocampus and no changes in the GR mRNA concentration (Lopez et al., 1998). Decreased MR mRNA has also been found in the hippocampus of neonatal rats subjected to maternal separation (Vázquez et al., 1996).
It has also been shown that polymorphisms of the angiotensin-converting enzyme (ACE) gene and angiotensin receptor gene have a predictive effect on treatment response to conventional antidepressants. The availability of an active ACE variant, indicating higher aldosterone levels, is associated with a poor therapy response (Bondy et al., 2005). This implies that the activity of the RAAS could be involved in the pathophysiology of depression.

Cortisol (and corticosterone in rodents) as a main part of the HPA axis plays an important part in the stress response of depression. Its concentration during awakening differs in different clinical depression subtypes, like melancholic depression and atypical depression from that of healthy subjects (Lamers et al., 2012b). Especially in melancholic depression increased plasma concentrations of cortisol have been reported consistently. High cortisol levels are considered to be a part of a deranged HPA axis activity in melancholic depression. Hypercortisolism is often also an indication of a more severe major depressive episode. This chronic 'fight-or-flight' situation is caused by a deranged HPA axis activity (Murck et al., 2014). In contrast, in atypical depression the HPA axis can be hypoactive: Lamers and colleagues showed in 2012 that patients with severe melancholic depression had significant higher morning cortisol levels than within the control group, whereas patients with atypical depression had a significant lower morning cortisol level than within the control group (Lamers et al., 2012b). These results point to different regulatory states of the HPA axis in different depression phenotypes. Summarized, aldosterone can possibly be altered in specific depression subtypes. The involvement of the RAAS in depression and in particular in depression refractory to standard treatment is in its early stages.

'Besides an increase in plasma aldosterone, neuroendocrine data point to a desensitization of peripheral MR in refractory depression: Challenge tests in patients with depression revealed no differences to healthy volunteers in milder un-medicated patients (A. H. Young et al., 1998), whereas in patients with therapy refractory depression a desensitization to the MR agonist prednisolone is apparent (Juruena et al., 2013; 2009). Because prednisolone does not cross the blood-brain barrier [(BBB)] in relevant amounts when administered in low doses (Karssen et al., 2002) the effect of prednisolone appears to act primarily at the pituitary. A further moderator of MR function [and treatment response] appears to be a history of early live stress, which leads to a reduced […] cortisol [awakening response (CAR)] and increased sensitivity for the suppression with prednisolone in comparison to patients without early live stress ([(Heim and Binder, 2011);](Werne Baes et al., 2013)). It has been suggested that the history of trauma and these biological characteristics may be related to clinical symptoms of atypical depression (Juruena et al., 2013; Murck et al., 2012; Werne Baes et al., 2013).' (Büttner et al. 2015: 25-26).

Besides the central MR sites, peripheral MR can be found in the kidney, adipose tissue, endothelium, macrophages, skin, lung and others (Martinerie et al., 2013). In the kidney, mostly in the distal convoluted tubules and in the cortical collecting duct of the distal nephron, the presence of 11ß-HSD_2 ensures specificity of aldosterone (Martinerie et al., 2013). If MR dysfunction is relevant in some forms of depression, peripheral MR could be affected as well.

This work is going to examine these systems. Therefore, to further evaluate the role of aldosterone and MR in depression this study uses functional parameters to characterize MR sensitivity in depression. The following part explains the underlying mechanisms of relevant functional parameters.

1.4. Markers of MR function

In order to characterize MR activity, MR-related biomarkers with proven technical validity were chosen. This paragraph explains the biological background motivation for the selection of the chosen MR-related biomarkers.

Functional biomarker parameters were used because it is not possible to directly measure central MR activity in humans. Besides the levels of cortisol and aldosterone, salt taste intensity (STI), and salt pleasantness (SP), heart rate variability (HRV) and

slow wave sleep (SWS) were used to characterize central MR activity. To measure the peripheral MR activity the electrolytes magnesium (Mg^{2+}), Na^{+}, and K^{+} and systolic blood pressure (SBP) were chosen. The separation of MR activity into central (inside CNS) and peripheral (outside CNS) biomarkers is referred to their main location involved in the mode of action of these respective biomarkers. These biomarkers of MR activity are examined in this work and set in relation to the treatment response with standard therapy in hospitalized patients with depression.

1. Salivary aldosterone and cortisol

'Aldosterone and cortisol are both ligands of the MR. Because MR activation suppresses HPA axis activity the ratio of aldosterone/cortisol [(aldo/cort)] constitutes a relevant functional marker (Buckley et al., 2007; Otte et al., 2003; Steiger et al., 1993).' (Büttner et al. 2015: 26).

To measure the two hormones saliva samples were chosen in order to establish a method that is easy to handle, collect, and less affected by the potential stress of sampling in comparison to blood draws.

2. Salt taste sensitivity

Administration of aldosterone increases Na^{+} intake linearly (Wolf and Handal, 1966). Vice versa, Na^{+} depletion is a strong physiological stimulus for aldosterone synthesis via the RAAS (Schweda, 2015). Aldosterone specifically affects the NTS in the brainstem, which is involved in salt appetite and salt taste (Licht et al., 2013; 2008). Because of synaptic connections with the NTS the nucleus accumbens is involved in salt-seeking behavior and is activated during salt intake (Shekhtman et al., 2007). Morphological and functional changes in the nucleus accumbens occur in response to Na^{+} depletion and increased aldosterone levels, which are similar to those observed after amphetamine sensitization (Roitman et al., 2002; Tandon et al., 2012). The nucleus accumbens gets strongly activated by sham-drinking during the expression of furosemide induced Na^{+} appetite in rats (Voorhies and Bernstein, 2006).

Salt intake is considered to protect from stress (Goldstein and Leshem, 2014) driven by central circuits enhancing the demand for salt intake. Plasma Na^{+} itself is directly related to cerebrospinal fluid Na^{+} (Doi et al., 1992) and seems to have an impact on Na^{+} appetite though no central Na^{+} sensing mechanism has been clearly detected yet (Geerling and Loewy, 2008).

Because salt liking drives salt intake a test for STI and SP can be regarded as a proxy for the central effect of aldosterone on the MR in these specific anatomical areas.

3. Heart rate variability

The HRV is a result of the balance between sympathetic and parasympathetic nervous system activity. Healthy cardiac activity is displayed by a high degree of beat-to-beat variability. Generated by autonomous reflexes the respiratory sinus arrhythmia (RSA) displays the parasympathetic function and is primarily mediated by the vagus innervation of the heart, which mainly mediates cardiac function (Mazzeo et al., 2011). In general, sympathetic activity is accelerating the heart rate, whereas parasympathetic activity is slowing down the heart rate. This difference is the basis for HRV as a marker of autonomic function.

Aldosterone stimulates increased sympathetic drive whereas spironolactone, a MR antagonist, reduces sympathetic drive in animal models (Francis et al., 2001; Grippo and Johnson, 2009). In humans, *'HRV is also associated with the activity of the […] RAAS (Ovaert et al., 2010; Schmidt et al., 1999) and MR activation (MacFadyen et al., 1997).'* (Büttner et al. 2015: 26).

A mechanism for the action of aldosterone on HRV could involve aldosterone sensitive neurons in the NTS (Shin et al., 2009). The vagus nerve arises from the brain stem and has connections to aldosterone sensitive sites in the NTS (Geerling and Loewy, 2009; Robson et al., 1998; Rottenberg, 2007), which relays into the sympathetic nervous system. In addition to the central regulation of HRV, cardiac function is modulated by autonomic reflexes during in- and expiration via pulmonary stretch-, cardiac mechanoreceptors and others which increase and decreases heart rate during in- and expiration, respectively (Ravits, 1997).

In patients with depression the autonomic nervous system is altered and shows an increased sympathetic drive (Udupa et al., 2007). *'It has been shown that RSA is reduced in patients with major depression compared to controls (Licht et al., 2013; 2008). A recent meta-analysis has demonstrated an association between increasing depression severity and lower HRV (Kemp et al., 2010; Licht et al., 2008).'* (Büttner et al. 2015: 26). Reduced HRV in depression can have its origin in predominant sympathetic tone, reduced parasympathetic tone or both. Several additional mechanisms are considered to activate the SNS in depression. For example, activation of HPA axis could increase sympathetic activity (Francis et al., 2001; 2003) via CRH

(Brown et al., 1982) and the locus ceoruleus that is influencing the SNS (Elam et al., 1986a; 1986b). Accordingly, chronic mild stress results in higher sympathetic tone (Grippo and Johnson, 2009). Overall, the data suggests that HRV, measured here by RSA, can be a valid marker for central MR activity.

4. Slow wave sleep

'The connection between sleep, depression and HPA axis activity has been described frequently (Murck et al., 2012; Thase et al., 2010). In particular, patients with high HPA axis activity appear to demonstrate lower SWS (Hubain et al., 1998). The MR antagonists spironolactone or its metabolite canrenoate suppress SWS (Born et al., 1991) and increase cortisol secretion (Heuser et al., 2000b). […] [C]ortisol increases SWS (Bohlhalter et al., 1997), [therefore,] an indirect sleep modulation effect of cortisol can be ruled out. […] [Hence], central MR activation may be regarded as SWS stimulating, i.e. higher SWS is a marker of central MR activation.' (Büttner et al. 2015: 26).

In depression SWS can be used to distinguish between patients with melancholic and patients with non-melancholic depression, especially amongst young and middle-aged men (Antonijevic, 2008) and therefore provide insights into MR function of an individual patient.

5. Electrolytes

'The classic physiological effect of aldosterone is peripheral electrolyte regulation at the level of the kidney. […] K^+ *and […]* Mg^{2+} *excretion is mainly regulated by aldosterone. Therefore, in primary aldosteronism* Mg^{2+} *and* K^+ *concentrations are decreased in serum and increased in urine excretion (Horton and Biglieri, 1962).'* (Büttner et al. 2015: 26). Furthermore, Horton and Biglieri showed that after adrenalectomy urinary K^+ and Mg^{2+} decreased. This effect was also prevalent after spironolactone administration in patients with primary aldosteronism (Horton and Biglieri, 1962). Mg^{2+} could be involved in the pathogenesis of depression (G. A. Eby and K. L. Eby, 2006; G. A. Eby et al., 2011) and has a close connection to the RAAS (Held et al., 2002). Further, its administration could also affect sleep (Murck and Steiger, 1998).

On the other hand, increased Na^+ uptake by MR activation takes place mostly in the gut and the proximal and distal nephron where it mediates aldosterone-stimulated Na^+

reabsorption through the epithelial Na^+ channel (ENaC) activation and the Na^+/K^+-ATPase (Viengchareun et al., 2007). As aldosterone contributes to the regulation of electrolytes it is an important regulatory mechanism to control plasma volume and blood pressure. Elevated aldosterone levels can stimulate an almost complete conservation of Na^+ from the urine (Geerling and Loewy, 2008). Besides its regulation at the kidney, aldosterone also regulates Na^+ concentration of saliva and sweat. Overall, Na^+, K^+ and a ratio of Na^+/K^+ are used in this study as markers of peripheral MR function.

6. Blood pressure

'[T]he blood pressure regulating effects of aldosterone are well established.' (Büttner et al. 2015: 26). In this work the focus lies on the SBP as additional and easily assessable biomarker. Blood pressure is mediated by many different mechanisms. One major regulatory part is the vegetative nervous system, i.e. sympathetic tone (Guyenet, 2006) as mentioned above in the section explaining HRV. Besides the vegetative nervous system, RAAS is particularly involved in patients suffering from depression and hypertension. In these patients elevated aldosterone levels possibly demand a different regime of pharmacologic antidepressant therapy (Hafner et al., 2013). The regulatory mechanism of blood pressure is closely related to MR but not yet completely understood (Geerling and Loewy, 2009; E. P. Gomez-Sanchez and C. E. Gomez-Sanchez, 2012). Other regulatory mechanisms of SBP involve catecholamines like norepinephrine (Gold et al., 2005).

Lenoir and colleagues reported in elderly depressed individuals lower systolic (3 mmHg) and diastolic (1 mmHg) blood pressure compared to non-depressive controls. This result stayed significant even after correction for heart failure and was independent of age and the use of antihypertensive or psychotropic agents (Lenoir et al., 2008; Scuteri, 2008). Hence it is important to note that depression and blood pressure are closely interconnected in a somewhat surprising way.

In this study SBP is considered a marker of peripheral MR activity.

1.5. Hypotheses and aim

Over the past few years a movement called the 'Quantified-Self' has gained importance amongst the general population (Spiegel Online 14.03.2013, ‚*Quantified-Self-Bewegung: Miss dich selbst*' by Hristio Boytchev; Süddeutsche Zeitung

13.01.2015, ‚*Das vermessene Ich*' by Jan Willmroth). Technological progress allows today to measure every movement and a number of biological functions of one's life through smart devices. These can be functions like the heart rate, diet or sleep. The collected data can be put into a wider picture and give the consumer suggestions for a healthier lifestyle. The increased availability of these self-measured biomarkers for the general public vastly increases the possibilities to understand biological processes to new extends. In the context of depression biomarkers could allow the patient to control his or her own progress during antidepressant treatment. Further, biomarkers could provide the clinician with information that could predict the effectiveness of a used psychotropic drug or even treatment response in general. Therefore, in the field of psychiatric research sufficient biomarkers have to be developed. Until today only few mostly experimental biomarkers exist to characterize treatment response and new strategies for their development have to be considered (Institute of Medicine, 2008; Murck et al., 2015). This work wants to contribute to the characterization of new possible biomarkers in depression, which focus on one identified functional system.

Three hypotheses are formulated to evaluate the influence of aldosterone and other biomarker parameters of central and peripheral MR function on the course of depression.

Hypothesis one evaluates the predictive value of baseline biomarker parameters for the relative change of depressive symptomatology within six weeks. It is hypothesized that higher aldosterone levels at baseline as well as central and peripheral MR hyperfunction may predict a poorer therapy response after six weeks.

Hypothesis two and three look at the plasticity of MR function displayed by its functional parameter change in the course of depression.

Hypothesis two evaluates the predictive value of the early change (within two weeks from baseline) of MR function towards the relative change of depressive symptomatology within six weeks. It is hypothesized that an early reduction of MR activity is predictive for a favorable relative change of depressive symptomatology within six weeks.

Finally, **hypothesis three** evaluates the late change (within six weeks from baseline) of MR function as a surrogate marker for the relative change of depressive symptomatology within six weeks, hypothesizing that unchanged MR activity indicates therapy refractoriness to standard antidepressant therapy.

By using the above mentioned fast and easy to use biomarker parameters for the assessment of MR functionality, the goal is to identify possible individualized patterns that could help later on to create more successful antidepressant therapy strategies and a new approach to personalized medicine for patients suffering from depression.

2. Materials and methods

2.1. Study collective

As described in my previous work: *'A total of […] [34] patients were included of which the main diagnosis was a single major depressive episode (ICD10: F32; n = 14), an episode of a recurrent major depression (F33; n = 18) or dysthymia (F34.1; none as primary diagnosis), as assessed by experienced clinicians and according to patient charts. All patients were in-patients at the Department of Psychiatry and Psychotherapy, Philipps-University Marburg, Germany.'* (Büttner et al. 2015: 26).

Patients were treated with standard care overall following the German National Disease Management Guideline/S3-Guidline for unipolar depression in a clinical setting specialized in depression.

'Nine of these subjects had an additional diagnosis of dysthymia (F34.1); six subjects with alcohol abuse F10.1; three subjects with nicotine dependence (F17.1); two subjects with somatoform disorder (F45); two subjects with an anxiety disorder (F41) and two subjects with insomnia (F51.0). All patients provided written informed consent. Patients were excluded if they had schizophrenia, delusional disorder, relevant neurological disease or severe internal diseases. Two [of the 34] patients were excluded from the analysis: One was diagnosed with multiple sclerosis in the course of the clinical stay and another reclassified with panic disorder as the main diagnosis.' (Büttner et al. 2015: 26-27).

Two of the remaining 32 participating patients could not be analyzed because of insufficient data at visit one, two and three. One of these two patients had to leave the clinic because of issues with political asylum. The other one withdrew informed consent before the baseline examination.

Therefore, in total 30 patients were participating. During the study problems with some of the patients occurred. Six patients dropped out because of multiple reasons. Two patients were uncooperative and therefore discharged, one patient left the clinic

against medical advice, one patient committed suicide and in two patients the late follow-up visit could not be carried out due to study staff vacation. '*24 patients completed all three study visits.*' (Büttner et al. 2015: 27).

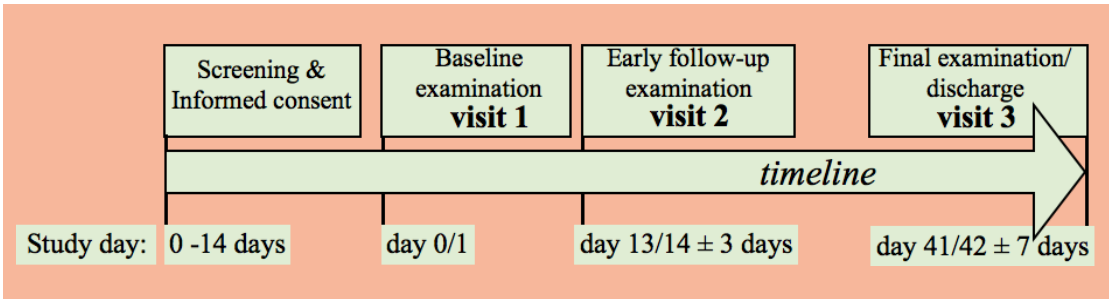
2.2. Study design

This study was designed as a six weeks' non-interventional follow-up study (Büttner et al., 2015). The sequence of examinations as well as the statistical analysis had been pre-defined in a study protocol. The study was approved by the Ethical Committee of the Medical Faculty of the Philipps-University Marburg.

After a screening period patients were examined three times during their clinical stay. Examinations took place at baseline, two weeks and finally six weeks after baseline (Büttner et al., 2015). *'In the case of discharge before week 6, the last examination was conducted earlier at the time of discharge'* (Büttner et al. 2015: 27). See Figure 2 for the study protocol timeline.

Figure 2: Study protocol timeline

Informed consent and screening were conducted in the first 14 days of patient's clinical stay. Baseline examination was conducted maximal 14 days after hospitalisation. The first follow-up examination was conducted approximately two weeks after baseline ± three days. Final Examination was conducted six weeks after baseline ± seven days.



'Clinician rating took place in the evening from around 4 to 8 pm […]. A sleep monitor was installed overnight. Read out followed the next morning. Testing for further central and peripheral MR parameters was also conducted the following morning [right after awakening and in the case of saliva sample collection] before patients got out of bed, […]' (Büttner et al. 2015: 27). These measurements included HRV, STI, SP, SBP and self-ratings scales such as the Quick Inventory of Depressive Symptomatology, self-rating with 16 items (QIDS-SR-16). '*Blood samples for the analysis of electrolytes were taken in the morning before noon after lying in supine positions for 30 min.*' (Büttner et al. 2015: 27). See Table 1 for a detailed overview of study examinations and assessments.

The duration of current depressive episode in weeks was assessed in retrospect. Also during the first study interview the age of onset of depression, the number of depressive episodes and the length of illness including the duration of the current depressive episode were assessed.

At every examination medication was assessed and coded into two main groups. The first group was categorized as medications having an impact on the CNS. They were subsumed into 7 categories: 1. SSRIs/SNRIs, 2. Mirtazapine, 3. MAO-inhibitors, 4. Atypical neuroleptics, 5. Lithium, 6. Tricyclic antidepressants, 7. All others, i.e. including typical neuroleptics and anticonvulsants. The second group was categorized as medication for somatic diseases. They were subsumed into 5 categories especially focusing on RAAS influencing compounds: 1. Medication affecting the RAAS, i.e. ß-blockers, renin inhibitors, ACE inhibitors and Angiotensin II receptor blockers (ARB), 2. Aldosterone antagonists, 3. Magnesium, 4. Glucocorticoids 5. All others.

Table 1: Overview of study examinations and assessments

This table gives an overview of study examinations and assessments carried out at every visit. Before the baseline examination resident clinicians confirmed clinical diagnosis, a short patient's history including family history was assessed, as well as socio-demographic characteristics, alcohol and nicotine consume. Medication and weight were assessed at all three visits. Hamilton Depression Rating Scale with 21 items (HDRS-21), Clinical Global Impression (CGI) and Global Assessment of Functioning (GAF) were assessed in the evening. Patients then had to wear a sleep EEG device overnight. At the following morning saliva samples were taken immediately after awakening. The heart rate variability (HRV), salt taste intensity (STI), salt pleasantness (SP) and systolic blood pressure (SBP) were assessed afterwards. Then patients had to fill out the Quick Inventory of Depressive Symptomatology, self-rating scale with 16 items (QIDS-SR-16) and the Beck Depression Inventory (BDI). Finally, blood samples for electrolytes determination were taken before noon after 30 min in supine position.

Study examinations and assessments	**Screening**	*Baseline examination* **Visit 1**	*Early follow-up examination* **Visit 2**	*Final/Late follow-up examination or discharge* **Visit 3**
Study day	0 - 14	Day 0 / 1	Day 13 / 14 ± 3	Day 41 / 42 ± 7 or discharge
Clinical diagnosis confirmation	X	X	X	X
Short patient's history incl. family history	X			
Assessment of socio-demographic features, alcohol and nicotine abuse	X			
Medication	X	X	X	X
Weight and Size	Size	Weight	Weight	Weight
HDRS-21, CGI, GAF		Evening (day 0)	Evening	Evening
Sleep EEG device		Overnight	Overnight	Overnight
Saliva sample, HRV, STI, SP & SBP		Morning (day 1)	Morning	Morning
QIDS-SR-16		Morning (day 1)	Morning	Morning
BDI		Morning (day 1)	-	Morning
Blood sample		Before noon	Before noon	Before noon

2.3. Saliva hormone measurement

'Morning saliva samples were taken […] immediately after awakening [at around 6:45 to 7:15 am]. Before getting up patients had to consecutively chew on two Salivettes® Cortisol (Sartstedt, Nümbrecht, Germany) in order to gain enough saliva. Samples were frozen at - 20°C up to two weeks and then at - 80°C until shipment by mail to the Institute of Experimental Endocrinology (Slovak Academy of Sciences, Bratislava, Slovakia) for analysis [by Daniela Jezova and colleagues].' (Büttner et al. 2015: 27). Study staff was blinded towards the concentrations of aldosterone and cortisol until the patient completed the trial.

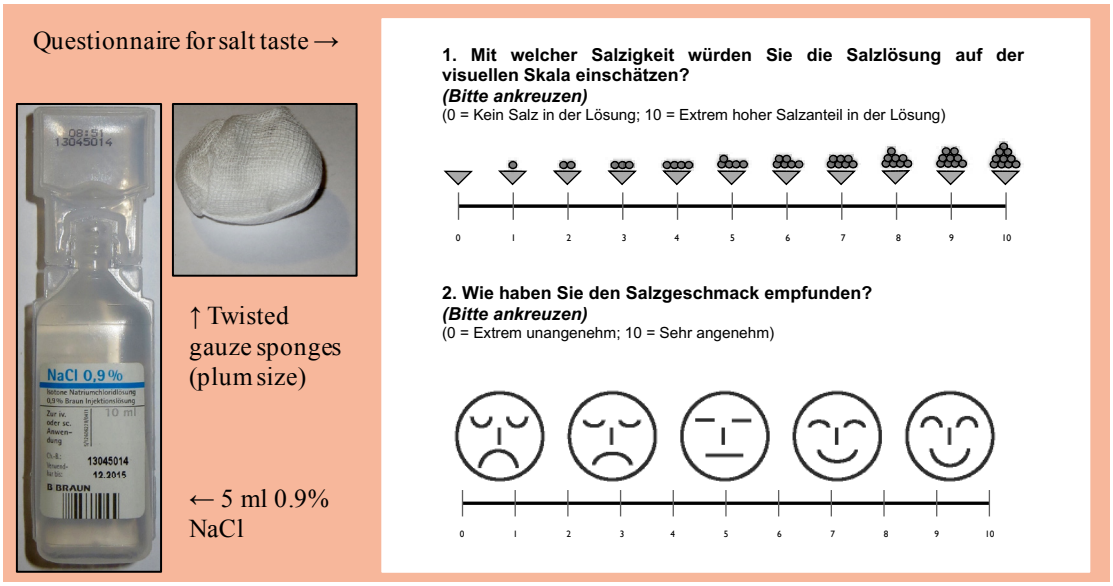
As described previously by Jezova and Hlavacova in 2008, saliva for the measurement of cortisol was analyzed with radioimmunoassay. Saliva aldosterone was concentrated three times and then measured with a modified direct radioimmunoassay for plasma aldosterone (Immunotech, Prague, Czech Republic) (Büttner et al., 2015; Hlavacova et al., 2013; Jezova and Hlavacova, 2008). However, physiological ranges for these salivary measurements are not well established for clinical practice.

2.4. Salt taste intensity and pleasantness

The evaluation of STI and SP based on the appraisal of a defined salt concentration was measured through a simple test. This test has been developed for the use in this trial (Büttner et al., 2015). *'Patients had to evaluate the salt taste of plum sized twisted gauze sponge (Pagasling®, Hartmann, Heidenheim, Germany) saturated with approximately 5 ml of a 0.9% sodium [chloride (NaCl)] solution (Mini-Plasco®, Braun, Melsungen, Germany) [(see left side of Figure 3)]. At every examination the same concentration and amount was used. Patients were blinded towards the concentration of the salt solution. They had to taste the solution in the gauze sponges for around 3 s and then had to spit it out.'* (Büttner et al. 2015: 27). To assess subjective STI and SP an 11-point Likert scale was used (see right side of Figure 3).

Figure 3: Methodology of the assessment of salt taste intensity and salt pleasantness

Patients had to fill out the questionnaire on the right side directly after tasting around 5 ml of a 0.9% sodium chloride (NaCl) solution in a twisted gauze sponges. The patients were blinded towards the concentration of the salt solution. The two questions evaluated the salt taste intensity (STI, question 1) and the salt pleasantness (SP, question 2) on 11-point Likert scales.



Picture source: Photographs on the left side by Matthias Braunisch

2.5. Heart rate variability (respiratory sinus arrhythmia)

The methodology of HRV measurement has been described in my previous work and is as follows:

'For measurement of […] [HRV] an easy to use smartphone app, combined with a Bluetooth receiver connected to two ECG electrodes (iThlete®, HRV Fit Ltd, Hampshire, United Kingdom) was utilized. The measurement took place 5 - 10 min after awakening. […] [HRV] was measured twice during a 55 s test interval each. Patients followed the breathing cadence of 7.5 breaths per min displayed by a lung animation on the smartphone screen. Calculations performed within the iThelete® app are based on the time domain of the HRV index, which is the root mean square of successive R-R-intervals (RMSSD). This measure does not appear to be influenced by breathing rates, unlike spectral indices, making it more suitable for quick clinical use (Penttilä et al., 2001). The RMSSD is respiratory sinus arrhythmia mediated and reflects parasympathetic cardiovascular modulation (Massin et al., 1999). [Therefore, it can be regarded as a measurement of RSA.] […] [The iThelete® app]

automatically modified [the RMSSD values] by taking the natural log transformation and multiplying it by twenty to provide a more intuitive value on a roughly 100 point scale (Flatt and Esco, 2013). External technical validation against standard ECG measures demonstrated satisfactory results ($r = 0.99$, $p < 0.001$) (Flatt and Esco, 2013).' (Büttner et al. 2015: 27).

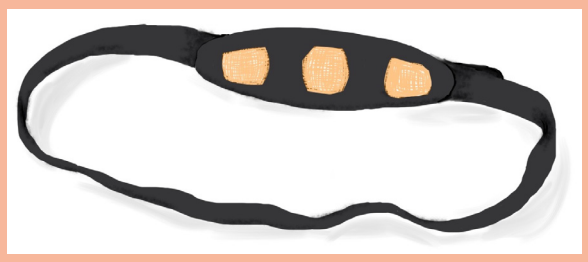
2.6. Slow wave sleep

'Sleep monitoring was carried out by a simplified polysomnographic system (Zeo™, Inc., Newton, MA, USA) that used only three proprietary sensors in a headband that transferred sleeping data wirelessly to a bedside monitor. The electrodes of the headband were placed on the forehead below Fp1, Fpz, Fp2 [(Figure 4)]. Scoring took place in 30 s […] [intervals] each night using four sleeping stages (wake, light sleep, deep sleep and rapid eye movement sleep). For more details see (Shambroom et al., 2012). The advantage of this monitoring system is that assessments are carried out in the usual sleeping environment, thus avoiding the common problems of sleep laboratory settings. However, validity [of the Zeo™ system], as determined by correlation with an expert system, has only [been] demonstrated for SWS and sleep duration, therefore […] [the analysis in this work was restricted] to SWS (Griessenberger et al., 2012).' (Büttner et al. 2015: 27).

The Zeo™ company provides as estimates for the average SWS duration for healthy subjects in their 20's 83 min, in their 30's 69 min, in their 40's 56 min, in their 50's 44 min, in their 60's 36 min and for consumers in their 70's 30 min.

Figure 4: Sleep monitoring

The wireless headband transferred polysomnographic data to a bedside monitor. The advantage of this monitoring system was, that patients were able to stay in their usual sleeping environment, thus avoiding the common problems of sleep laboratory settings. The electrodes relevant for this work are highlighted with 1 (Fp1), z (Fpz) and 2 (Fp2) in the left drawing.



↑ Headband with 3 sensors to place below Fp1, Fpz and Fp2.

← Adjustable and wireless headband with a wireless connection to a bedside monitor. The electrodes Fp1, Fpz and Fp2 are marked with 1, z and 2.

Image source: Drawings by Matthias Braunisch modified after the Zeo™ user manual.

2.7. Electrolytes and blood pressure

'Plasma Na^+ and K^+ levels were measured using specific ionic selective electrodes. Mg^{2+} [ions] were measured photometrically at 520 nm after complexion with calmagite (3-Hydroxy-4-[(2-hydroxy-5-methylphenyl)azo]-1-naphthalenesulfonic acid).' (Büttner et al. 2015: 27). The Institute of Laboratory Medicine of the Philipps-University Marburg conducted these routine measurements. This institute quoted the physiological range of the mentioned electrolytes as follows: Mg^{2+} 0.65 – 1.05 mmol/l, Na^+ 135.0 – 145.0 mmol/l and K^+ 3.4 – 4.5 mmol/l.

'*Blood pressure was measured using an automatic blood pressure monitor (Omron M5 Professional™, Omron Medizintechnik, Mannheim, Germany) mounted on patient's upper right arm.*' (Büttner et al. 2015: 27).

A physiological systolic blood pressure was assumed between 100 and 139 mmHg.

2.8. Clinical outcome parameters

'One trained rater (M.B.) [the author of this work, Matthias Braunisch] conducted all clinical ratings to avoid inter-rater variability.' (Büttner et al. 2015: 27). He was trained and supervised over a one-month period in the clinical rating scales. Clinician rating was conducted in the evening before sleep monitoring using the Hamilton Depression Rating Scale with 21 items (HDRS-21) (Hamilton, 1960) (Büttner et al., 2015).

'To focus on the core symptoms of depression the one-dimensional HDRS-6 subscale (Bech et al., 1975) was used […] [as primary outcome measure] as it is considered to measure more accurately the […] [pure] antidepressant effects (Bech, 2006; Lecrubier and Bech, 2007).' (Büttner et al. 2015:27).

The HDRS-6 contains the following items: 1. Depressed mood, 2. Work and interest, 3. General somatic (tiredness), 4. Psychic anxiety, 5. Guilt feelings, 6. Psychomotor retardation.

Besides the HDRS-21, the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) and the Clinical Global Impression Scale (CGI, Guy W., 1976) were assessed in the evening.

'For the assessment of anxiety […] item no. 10 (anxiety-psychic) of the HDRS-21 [was used].

The morning after sleep monitoring, patients rated several aspects of psychopathology themselves using the Beck Depression Inventory (BDI) (A. T. Beck et al., 1961) and […] QIDS-SR-16 (Rush et al., 2003), which is of particular interest as it contains information on depressive subtypes, like atypical depression symptoms (Murck, 2003).' (Büttner et al. 2015: 27-28).

Because of the potential link between biological features and subtypes of depression (Lamers et al., 2012a), subtypes were pre-specified based on vegetative features in analogy to Mannel and colleagues (Mannel et al., 2010). Patients were then assigned to the three subtypes of depression, i.e. melancholic, atypical or non-vegetative depression, according to their QIDS-SR-16 score at baseline. The vegetative features were: early morning awakening versus hypersomnia (item no. 3 and 4), loss of appetite versus increased appetite (item no. 6 or 7) and weight loss versus gain (item no. 8 or 9). In each of these three items zero to three points could be reached.

The categorization followed the following steps: in a first step patients with vegetative melancholic features were identified. This was the case if at least one of the QIDS-SR-16 items had a value of ≥ 2 in: either 1) item no. 3, waking up too early; 2) item no. 6, decreased appetite; or 3) item no. 8, weight loss.
In a second step depression with atypical features was identified. This was the case if at least one item had a value ≥ 2 in: either 1) item no. 4, sleeping too much; 2) item no. 7, increased appetite; or 3) item no. 9, weight gain.
If patients didn't meet neither the criteria for melancholic nor atypical depression they were categorized as patients with non-vegetative depression.

2.9. Statistical analysis

'Statistical analysis was planned prior to the study in a study protocol. According to the study protocol, the primary objective […] was to investigate the correlation of the defined biomarker parameters [(at baseline, their early and late change)] with the […] [relative change of depressive symptomatology after six weeks] of patients with depression treated with standard care.' (Büttner et al. 2015: 28).
Response of clinical symptoms was considered as a final (visit three) HDRS-6 reduction of 50% compared to baseline. A final HDRS-21 score of ≤ 7 at visit three indicated remission of clinical symptoms. To evaluate gender differences for response and remission a chi-square test was used.
To evaluate the influence of age on patients' biomarker parameter characteristics (dependent variable) an univariate analysis of variance (ANOVA) was performed with the inter-subject factors gender, depressive subtype and age as covariate.

For all three hypothesizes the impact of aldosterone, i.e. its baseline value, its early and late change from baseline, on the clinical outcome, as measured by the relative change of HDRS-6, was considered as primary analysis. '*Analyses on the basis of other biomarker parameters were considered secondary.*' (Büttner et al. 2015: 28).
The clinical outcome was defined as the proportional change relative to the baseline value of the clinical rating scales (i.e. 100% corresponds to no change, 50% corresponds to a 50% reduction of severity after six weeks from baseline, etc.). As pre-specified in the study protocol the primary outcome variable was the proportional change of HDRS-6 relative to the baseline value. For all three hypotheses Pearson's

correlation coefficient (PCC) was used to measure the correlation between clinical outcome and the selected biomarkers (Büttner et al., 2015).

'*For all analyses gender was considered as a stratifying factor.*' (Büttner et al. 2015: 28). Therefore, all analyses were performed separately for the total group (i.e. including male and female patients), for male and for female patients. All parameters were considered to express MR function therefore co-linearity was assumed. Because of the assumption of multi-co-linearity, no correction for multiple testing was carried out.

For **hypothesis one** the relationship between each baseline biomarker parameter value and clinical outcome was correlated using PCC, i.e. the predictive value of baseline conditions were tested.

For hypothesis two and three the proportional change of the biomarker parameters from baseline to visit two (hypothesis two) and visit three (hypothesis three) were set into relation with clinical outcome.

Hypothesis two focused on early biomarker changes as predictors for the clinical outcome. To calculate the early change of biomarker parameters for hypothesis two the biomarker parameter value of visit two was divided by its baseline value (i.e. 100% corresponds to no change, 50% corresponds to a 50% reduction and 120% corresponds to a 20% increase of its baseline parameter activity, etc.). Then this proportional value, representing the early biomarker parameter change, was correlated with clinical outcome using PCC.

Hypothesis three examined the correlation of the late changes of the baseline biomarker parameters and the clinical parameters, i.e. biological correlates of response or surrogate markers. Similarly to hypothesis two, the late change of biomarker parameters was calculated for hypothesis three. For this, the biomarker parameter value of visit three was divided by its baseline value (i.e. 100% corresponds to no change, 50% corresponds to a 50% reduction and 120% corresponds to a 20% increase of its baseline parameter activity, etc.). Then, similar to hypothesis two, this proportional value, indicating late biomarker parameter change, was correlated with clinical outcome using PCC.

Different types of medication were summarized in groups as described above (Chapter 2.2. Study design, page 23). To evaluate possible confounders, the influence of RAAS modifying medication was tested in a general linear model with ANOVA

for each biomarker parameter at baseline, their early and late plasticity. Additionally, intake versus no intake of RAAS modifying medication (i.e. ß-blockers, renin inhibitors, ACE inhibitors, ARBs) was used as stratifying factor to further evaluate the influence of the biomarkers onto clinical outcome in patients free of RAAS modifying medication. However, a further separation into male and female patients, respectively, free of RAAS modifying medication then made the sample groups too small for a meaningful analysis. To evaluate the effect of the intake of medication over time repeated measures ANOVA was performed.

Additionally, to the pre-specified analysis of the study protocol exploratory analysis was carried out. *'For […] [baseline biomarker parameters,] which showed a significant correlation with […] [clinical outcome a] further exploratory analysis [was performed], splitting the data into two groups of patients with "high" and "low" marker values using the sample median of the biomarker [of patients who completed all three visits to descriptively compare the absolute values of depressive symptomatology] between these two groups.'* (Büttner et al. 2015: 28)
This was performed similarly for the early and late changes of biomarker parameters (hypotheses two and three). Here the data was divided into two groups of patients with 'reducing' and 'increasing' marker values. Markers below and including 100% were assigned to the 'reducing group' whereas markers above 100% were assigned to the 'increasing group' to descriptively compare the absolute values of depressive symptomatology between these two groups.
Only for aldosterone a split into three groups, i.e. 'reduction', 'unchanged' and 'increase', was performed. A stable aldosterone concentration was assumed when its value stayed in a ± 10% range compared to baseline. Below 90% a 'reduction' and above 110% an 'increase' compared to baseline was assumed.
Further, exploratory analysis was performed with the proportional clinical changes relative to baseline using the scores of HDRS-21, QIDS-SR-16 and BDI (i.e. accordingly to HDRS-6, 100% corresponds to no change, 50% corresponds to a reduction of depressive symptomatology of 50% compared to baseline, etc.). The relative change of these clinical measures was then correlated with the biomarker parameter at baseline, their early and late change using PCC.

In cooperation with the Institute for Medical Biometry and Epidemiology of the Philipps-University of Marburg sample size was calculated with 82 patients with an alpha of 0.05 to reach a statistical power of 80%. As in this study only 24 patients could be analyzed the present work represents a preliminary analysis of an ongoing study. The statistical analysis of the study protocol including the sample size calculation was designed with the help of Dr. Sebastian Irle. Statistical analysis was done with support from Brandon Greene, both members of the Institute for Medical Biometry and Epidemiology.

'For data collection Microsoft® Excel 2011 for Mac was used and then transferred to [Statistical Package for the Social Sciences®] SPSS[®]. For all statistical analysis [and graph depiction] SPSS[®] version 22 was used. Results were considered significant at a p-value < 0.05.' (Büttner et al. 2015: 28). Results with p-values of < 0.1 were considered as trends and are also reported.

3. Results

3.1. Demographic and descriptive data

3.1.1. Patient characteristics

Table 2 summarizes patient characteristics that completed all three study visits, differentiated by gender. Male patients had a mean age of 45.1 ± 14.3 (mean ± standard deviation), female patients were 43.9 ± 19.2 years old. Minimum age was 19 in male and 18 years in female patients. Maximum age was 73 in male and 75 years in female patients. In total 41.7% of the study patients were classified with melancholic, 33.7% with atypical and 25.0% with non-vegetative depression. There were no significant differences between genders (Büttner et al., 2015).

Calculations for BMI showed slight overweight in the total group with 27.4 ± 5.2 kg/m^2.

For visit one data of 30 patients was available (17 (56.7%) male, 13 (43.3%) female). Due to dropouts 28 patients (16 (57.1%) male, 12 (42.9%) female) for visit two and 24 patients (13 (54.2%) male, 11 (45.8%) female) for visit three remained for analysis. Dropouts were not included in descriptive analysis. Reasons for dropouts see Chapter 2.2. Study design (page 23).

Mean depression scores on HDRS-6 were 10.1 ± 2.8 for visit one, 7.3 ± 4.5 for visit two and 5.8 ± 4.0 for visit three. ANOVA with gender as inter-subject variable revealed no significant gender difference of HDRS-6 scores. For more details on the depression rating scores of HDRS-21, QIDS-SR-16 and BDI see Table 2.

In this study sample response rate was overall 50.0% (61.5% for men, 36.4% for women) and remission rate was overall 33.3% (46.2% for men, 18.2% for women). Chi-square test showed no significant gender difference, neither for response or remission.

Patients that completed all three study visits had a mean duration of depressive episode of 30.4 ± 55.0 weeks. The mean length of lifetime illness was 64.6 ± 111.1 weeks. The mean number of depressive episodes was 2.6 ± 2.0. The mean age of patients' first depressive episode was 36.6 ± 15.7. The mean duration of treatment for the current episode was 65.5 ± 27.9 days. Table 2 summarizes further information like minimum and maximum values of patients' disease history. ANOVA with gender as inter-subject variable did not demonstrate any significant influence on the criteria of disease history in this study sample.

Table 2: Patient characteristics

The table has been modified after Büttner and colleagues 2015. Characteristics of the 24 patients that completed all three study visits are shown separated by gender and in total including mean ± standard deviation (SD), minimum (min) and maximum (max). Depression subtypes were differentiated into melancholic depression (MD), atypical depression (AD) and non-vegetative depression (NV). Body mass index (BMI) was calculated. Mean clinical rating scale values of depressive symptomatology are shown on Hamilton Depression Rating Scale with 6 and 21 items (HDRS-6 & -21), Quick Inventory of Depressive Symptomatology, self-rating with 16 items (QIDS-SR-16) for all three visits and Beck Depression Inventory (BDI) for baseline and visit three. Response on HDRS-6 and remission on HDRS-21 of depressive symptomatology are shown. Mean, minimum and maximum values are shown for: duration of current depressive episode and length of illness in weeks; number of depressive episodes and age of onset of depression. Available data for analysis for baseline (visit one), visit two and three is shown. No significant gender differences existed for the characteristics of patients.

Patient characteristics	Male (n = 13)	Female (n = 11)	Total (n = 24)
Age (mean ± SD / min / max in years)	45.1 ± 14.3 / 19 / 73	43.9 ± 19.2 / 18 / 75	44.5 ± 16.4 / 18 / 75
Subtype (MD / AD / NV), N (%)	7 (53.8) / 4 (30.8) / 2 (15.4)	3 (27.3) / 4 (36.4) / 4 (36.4)	10 (41.7) / 8 (33.3) / 6 (25.0)
BMI at baseline (mean ± SD / min / max in kg/m^2)	27.5 ± 5.1 / 19.1 / 34.9	27.0 ± 5.6 / 20.1 / 37.1	27.4 ± 5.2 / 19.1 / 37.1
Depression scores			
HDRS-6 visit 1 / 2 / 3 (mean ± SD)	9.5 ± 3.2 / 6.8 ± 5.1 / 5.2 ± 4.5	10.8 ± 2.1 / 8.0 ± 4.0 / 6.5 ± 3.4	10.1 ± 2.8 / 7.3 ± 4.5 / 5.8 ± 4.0
HDRS-21 visit 1 / 2 / 3 (mean ± SD)	19.9 ± 6.4 / 14.1 ± 8.6 / 10.3 ± 7.6	22.3 ± 6.3 / 16.7 ± 5.6 / 13.6 ± 7.1	21.0 ± 6.3 / 15.3 ± 7.4 / 11.8 ± 7.4
QIDS-SR-16 visit 1 / 2 / 3 (mean ± SD)	12.5 ± 4.8 / 9.0 ± 5.5 / 7.4 ± 6.7	12.6 ± 5.8 / 10.4 ± 3.9 / 8.6 ± 5.1	12.6 ± 4.9 / 9.6 ± 4.8 / 7.9 ±6.0
BDI visit 1 / 3 (mean ± SD)	17.0 ± 11.2 / 11.3 ± 12.6	21.1 ± 8.5 / 13.4 ± 7.6	18.9 ± 10.1 / 12.3 ± 10.5
Response and remission			
Number of response on HRDS-6, N (%)	8 (61.5)	4 (36.4)	12 (50.0)
Number of remission on HRDS-21, N (%)	6 (46.2)	2 (18.2)	8 (33.3)
Disease history			
Duration of current depressive episode (mean ± SD / min / max in weeks)	35.1 ± 74.3 / 3.4 / 275.0	24.7 ± 16.2 / 4.4 / 60.0	30.4 ± 55.0 / 3.4 / 275.0
Length of illness (mean ± SD / min / max in weeks)	55.3 ± 114.6 / 9.0 / 431.6	78.2 ± 111.0 / 9.0 / 365.4	64.6 ± 111.1 / 9.0 / 431.6
Numbers of depressive episodes (mean ± SD / max)	2.4 ± 2.4 / 10	2.8 ± 1.9 / 8	2.6 ± 2.2 / 10
Age at onset (mean ± SD / min / max in years)	39.7 ± 11.9 / 19 / 58	33.0 ± 19.3 / 14 / 74	36.6 ± 15.7 / 14 / 74
Duration of treatment (mean ± SD / min / max in days)	58.2 ± 19.4 / 24 / 86	74.2 ± 34.4 / 29 / 147	65.5 ± 27.9 / 24 /147
Available data			
Available data for all 3 visits, N (%)	13 (54.2)	11 (45.8)	24
Available data for visit 1 & 2, N (%)	16 (57.1)	12 (42.9)	28
Available data for visit 1, N (%)	17 (56.7)	13 (43.3)	30

3.1.2. Biomarker parameter characteristics

Table 3 gives a descriptive overview of the biomarker parameter characteristics at baseline and overall (visit one, two and three).

The physiological range of saliva aldosterone and cortisol is not very well established for clinical practice. Concerning the electrolytes plasma Mg^{2+} values were in the physiological range. At baseline 1 (4.3%) and throughout the study 1 (1.4%) patient (the same as at baseline) had mild hyponatremia. No patient had hypernatremia. At baseline 2 (8.6%) and throughout the study 4 (5.6%) patients had hypokalemia and at baseline no and throughout the study 1 (1.4%) patient had a slightly hyperkalemic value.

At baseline no and throughout the study 1 (1.4%) patient had slight systolic hypotension and at baseline 7 (29.4%) and throughout the study 17 (23.8%) assessments showed systolic hypertension. Two patients had severe systolic hypertension at baseline. Their SBP was 182 mmHg and 185mmHg, respectively.

Table 3: Characteristics of biomarker parameters

This table gives a descriptive overview of median, mean ± standard deviation (SD), minimum and maximum of the biomarker parameters at baseline and pooled across all visits (visit one, two and three).

Characteristics of biomarker parameters	Median	Mean ± SD	Minimum	Maximum
Aldosterone (pg/ml)				
Baseline (n = 18)	4.1	11.6 ± 15.4	0.2	52.7
Pooled across all visits (n = 51)	3.9	9.6 ± 12.3	0.2	52.7
Cortisol (ng/ml)				
Baseline (n = 20)	4.3	5.5 ± 3.2	1.8	13.7
Pooled across all visits (n = 59)	4.4	5.9 ± 3.8	1.4	20.0
Aldosterone/cortisol ratio				
Baseline (n = 17)	$0.9*10^{-3}$	$1.9*10^{-3} \pm 2.2*10^{-3}$	$0.0*10^{-3}$	$7.0*10^{-3}$
Pooled across all visits (n = 47)	$0.9*10^{-3}$	$2.2*10^{-3} \pm 3.4*10^{-3}$	$0.0*10^{-3}$	$15.5*10^{-3}$
Central markers of MR function				
Salt taste intensity				
Baseline (n = 24)	3.3	3.8 ± 2.3	0.0	9.0
Pooled across all visits (n = 72)	4.0	4.2 ± 2.3	0.0	9.5
Salt pleasantness				
Baseline (n = 24)	5.0	4.8 ± 1.9	1.0	9.0
Pooled across all visits (n = 72)	5.0	4.5 ± 2.3	0.0	10.0
Heart rate variability				
Baseline (n = 21)	64.9	63.8 ± 14.2	42.0	88.4
Pooled across all visits (n = 64)	63.1	63.8 ± 2.3	35.1	102.5
Slow wave sleep (min)				
Baseline (n = 24)	38.5	44.5 ± 22.5	17.0	94.0
Pooled across all visits (n = 69)	43.0	47.2 ± 25.6	7.0	116.0
Peripheral markers of MR function				
Magnesium (mmol/l)				
Baseline (n = 23)	0.84	0.84 ± 0.05	0.76	0.94
Pooled across all visits (n = 69)	0.84	0.84 ± 0.05	0.71	0.96
Sodium (mmol/l)				
Baseline (n = 23)	138.0	138.7 ± 1.9	134.0	142.0
Pooled across all visits (n = 69)	139.0	138.8 ± 1.7	134.0	142.0
Potassium (mmol/l)				
Baseline (n = 23)	3.7	3.8 ± 1.9	3.2	4.4
Pooled across all visits (n = 69)	3.8	3.8 ± 0.3	3.0	4.6
Na^+/K^+ ratio				
Baseline (n = 23)	37.5	37.2 ± 3.3	31.4	42.8
Pooled across all visits (n = 69)	36.8	37.0 ± 3.2	30.0	46.3
Systolic blood pressure (mmHg)				
Baseline (n = 24)	127.5	132.2 ± 22.1	101.0	185.0
Pooled across all visits (n = 72)	127.0	129.6 ± 21.1	98.0	185.0

3.1.3. Medication

Repeated measures ANOVA revealed a stable intake of all categories of medication over the clinical stay except for somatic medication of the category 5 (all others) that was by trend different throughout the clinical stay ($p = 0.050$, $n = 24$).

The following CNS medication was taken by study participants and summarized under category 7 (all others): melperone, bupropion, lamotrigine, L-dopa/benserazide, maprotiline, methylphenidate, agomelatine, moxonidine, tizanidine, lorazepam and zopiclone.

The following somatic medication was taken by study participants and summarized under category 5 (all others): L-thyroxine, ethinyl-estradiol/chlormadinone, ethinyl-estradiol/levonorgestrel, pantoprazole, ranitidine, amlodipine, simethicone, hydrochlorothiazide, potassium/magnesium/folic acid/vitamin B_{12}/niacin/coenzyme Q_{10} (Tromcardin® complex), vitamin B_{12}, folic acid/ vitamin B_6/ vitamin B_{12} (Folplus®), vitamin A/ vitamin B_1 / vitamin B_2/ vitamin B_6/ vitamin B_{12}/ biotin/ folic acid/ niacin/ pantothenic acid/ vitamin C/ vitamin D3/ vitamin E/ vitamin K1/ calcium/ potassium/ magnesium/ chloride/ phosphor/ chromium/ iron/ iodine/ copper/ melybdenum/ selenium/ zinc (A-Z KOMPLEX-ratiopharm®), ursodeocycholic acid, allopurinol, simvastatin, diclofenac, paracetamol, metamizole, clonidine, acetylsalicylic acid, phenprocoumon, sitagliptin/metformin, valeriana/humulus (Luvased®), potassium effervescent tablet, flecainide, finasteride, ofloxacin, ceterizine and terazosin.

'*The three patients taking lithium and/or glucocorticoids took them during all three visits. In the whole study group and during all visits neither aldosterone antagonists nor MAO-Inhibitors had been prescribed.*' (Büttner et al. 2015: 28).

For more information on which medication was taken at baseline, visit two and three see Table 4.

Table 4: Medication

This table shows the intake of medication at baseline/visit one, visit two and visit three and has been modified after Büttner and colleagues 2015. The p-value (Greenhouse-Geisser) is given for repeated measures ANOVA, showing that the intake of medication of all categories except for the somatic category 5 (all others) was stable throughout the clinical stay. ß-Blockers, renin inhibitors, ACE inhibitors and ARBs were summarized as medication affecting the RAAS.

Medication, N (%)	Visit 1	Visit 2	Visit 3	P-value
Central nervous system medication:				
1. SSRIs/SNRIs	19 (79.2)	20 (83.3)	21 (87.5)	0.53
2. Mirtazapine	5 (20.8)	8 (33.3)	9 (37.5)	0.21
3. MAO-inhibitors	0	0	0	-
4. Atypical neuroleptics	4 (16.7)	7 (29.2)	8 (33.3)	0.16
5. Lithium	3 (12.5)	3 (12.5)	3 (12.5)	-
6. Tricyclic antidepressants	0	0	1 (4.2)	0.33
7. All others	9 (37.5)	8 (33.3)	7 (29.2)	0.44
Somatic medication:				
1. Medication affecting the RAAS	9 (37.5)	10 (41.7)	11 (45.8)	0.23
2. Aldosterone antagonists	0	0	0	-
3. Magnesium	2 (8.3)	4 (16.7)	3 (12.5)	0.23
4. Glucocorticoids	2 (8.3)	2 (8.3)	2 (8.3)	-
5. All others	9 (37.5)	7 (29.2)	12 (50.0)	0.05

3.2. Results hypothesis one: Outcome in relation to baseline markers

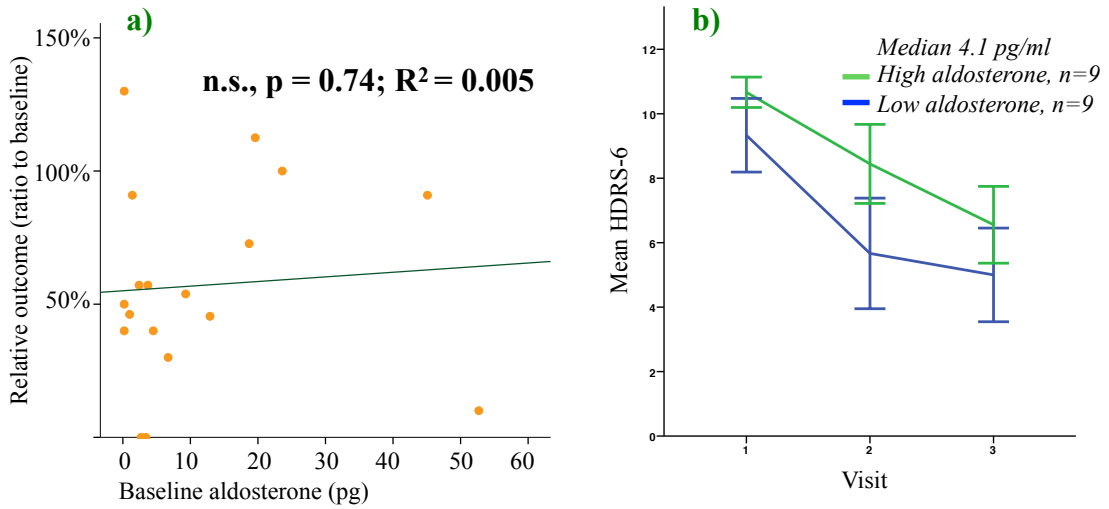
Hypothesis one examines the correlation of baseline biomarker parameter activity and the proportional change of depressive symptomatology after six weeks relative to baseline. It therefore examines the predictive value of a baseline biomarker parameter in terms of clinical outcome after six weeks. Table 5 gives an overview of the results of hypothesis one. Results of the relation between baseline markers and outcome have already been published (Büttner et al., 2015) and are presented in this work in a modified form.

3.2.1. Aldosterone and cortisol

At baseline, neither saliva aldosterone ($p = 0.74$) nor cortisol ($p = 0.66$) concentration correlated with the main outcome variable, the proportional change of HDRS-6 score (Figure 5 a) and b)).

Figure 5: Baseline aldosterone and outcome

In graph a) on the left side and in successive figures the x-axis represents the baseline value of the selected biomarker parameter. The y-axis represents the proportional change of the Hamilton Depression Rating Scale with 6 items (HDRS-6) from baseline to six weeks after baseline (i.e. 100% corresponds to no change, 50% corresponds to a 50% reduction and 120% corresponds to a 20% increase of the baseline HDRS score, etc.), i.e. it represents a relative value for antidepressant response. For each graph coefficients of determination (R^2) and p-values are indicated. Not significant depictions are indicated by n.s..
In graph b) on the right side and in successive figures the x-axis represents each visit. Graphs of the right side are strictly on a descriptive level to help visualize the data. Visit one is the baseline visit; visit two took place two weeks after baseline and visit three six weeks after baseline. The y-axis represents the mean absolute value of the HDRS-6. Error bars represent ± 1 standard errors of mean. The 24 patients that completed all study visits were split at the median into two groups according to their baseline parameter value. The value of the median itself was part of the low parameter group.

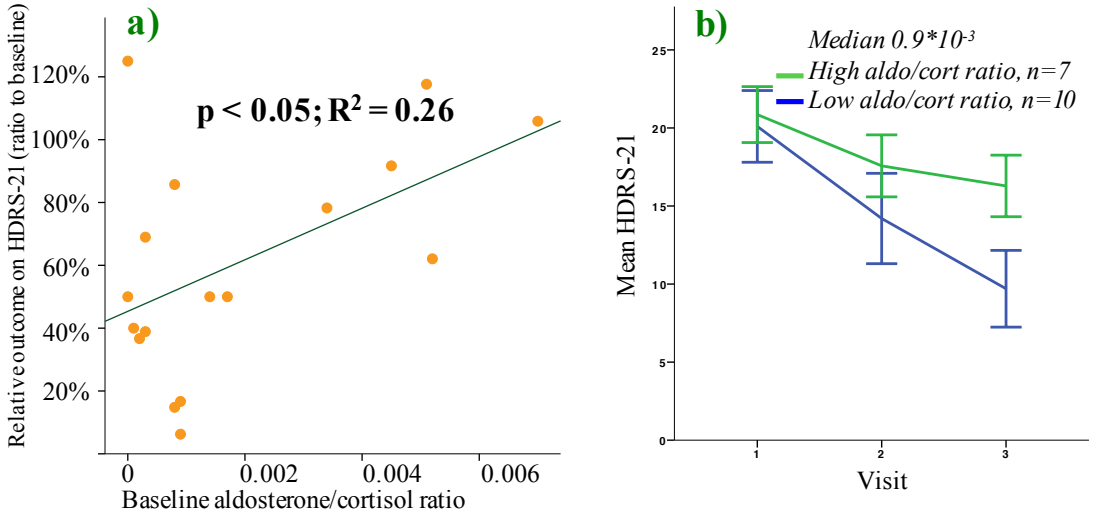


The aldo/cort ratio, as a marker of RAAS versus HPA activation, was by trend predictive for the relative change in the HDRS-6 response after six weeks in the total group ($p < 0.1$) and in female patients ($p < 0.1$).

In the additional analysis higher aldo/cort was predictive for poorer improvement after six weeks on HDRS-21 ($p < 0.05$) (Figure 6 a) and b)) (Büttner et al., 2015). Lower baseline aldosterone values were predictive for improvement after six weeks in the total group ($p < 0.01$) and in female patients ($p < 0.01$) on the proportional change of BDI (Büttner et al., 2015).

Figure 6: Ratio of aldosterone to cortisol in male subjects and outcome on HDRS-21

For explanations of the graphs of the left and right column, x- and y-axis see Figure 5. This figure has been modified after Büttner and colleagues 2015. Different to Figure 5 the proportional change of depressive symptomatology is here on Hamilton Depression Rating Scale with 21 items (HDRS-21).
Figure a) shows, that a ratio of aldosterone to cortisol (aldo/cort) at baseline was significantly correlated with relative outcome after six weeks on HDRS-21. This indicated that patients with higher aldosterone levels in relation to cortisol (i.e. high aldosterone and low cortisol levels) had a more severe depressive symptomatology after six weeks than patients with a lower ratio. This is shown on a descriptive level in figure b) for a group split at the median (0.9) (Büttner et al., 2015).



3.2.2. Central MR markers

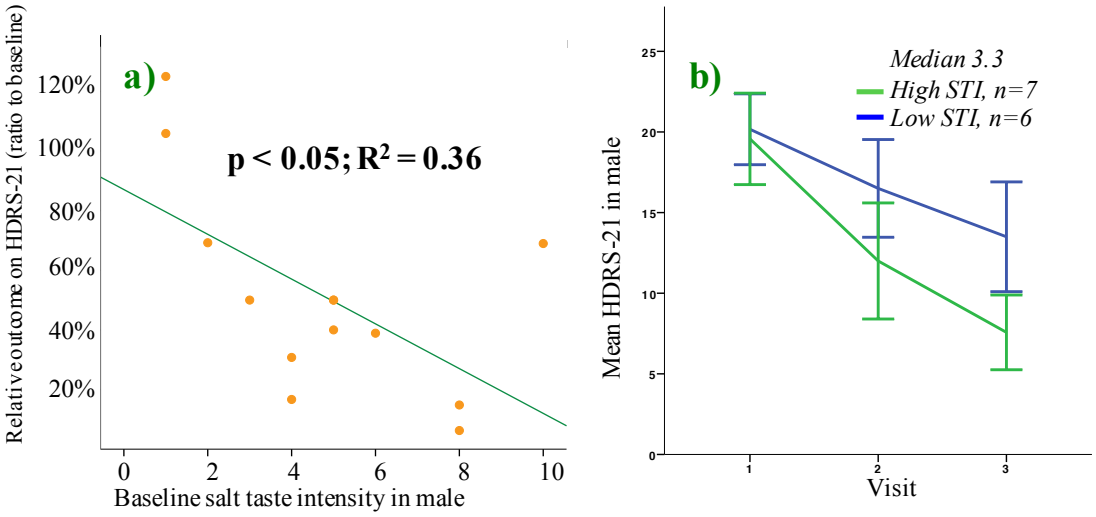
3.2.2.1. Salt taste intensity and salt pleasantness

In the total group patients with lower baseline SP improved by trend after six weeks on HDRS-6 ($p < 0.08$), this improvement was significant for male patients ($p < 0.05$) (Figure 8 a) and b)). No such correlation was found for STI ($p = 0.15$) and the relative change of HDRS-6.

'In male patients higher STI ($p < 0.05$) as well as lower SP ($p < 0.05$) at baseline was predictive for a better response on the HDRS-21 after 6 weeks ($p < 0.05$) […].' (Büttner et al. 2015: 29) (Figure 7 a) and b)) and by trend for the total group ($p < 0.09$).

Figure 7: Salt taste intensity in male subjects and outcome on HDRS-21

This figure has been modified after Büttner and colleagues 2015. For explanations of the left and right column, x- and y-axis see Figure 5. Different to Figure 5 the proportional change of depressive symptomatology is here on Hamilton Depression Rating Scale with 21 items (HDRS-21).
Figure a) shows, that higher baseline salt taste intensity (STI) of a defined 0.9% NaCl solution in male patients representing salt appetite (see also Figure 3), was associated with better depressive symptomatology after six weeks compared to baseline. This is shown on a descriptive level for a group slit at the median in figure b) (Büttner et al., 2015).



3.2.2.2. Heart rate variability

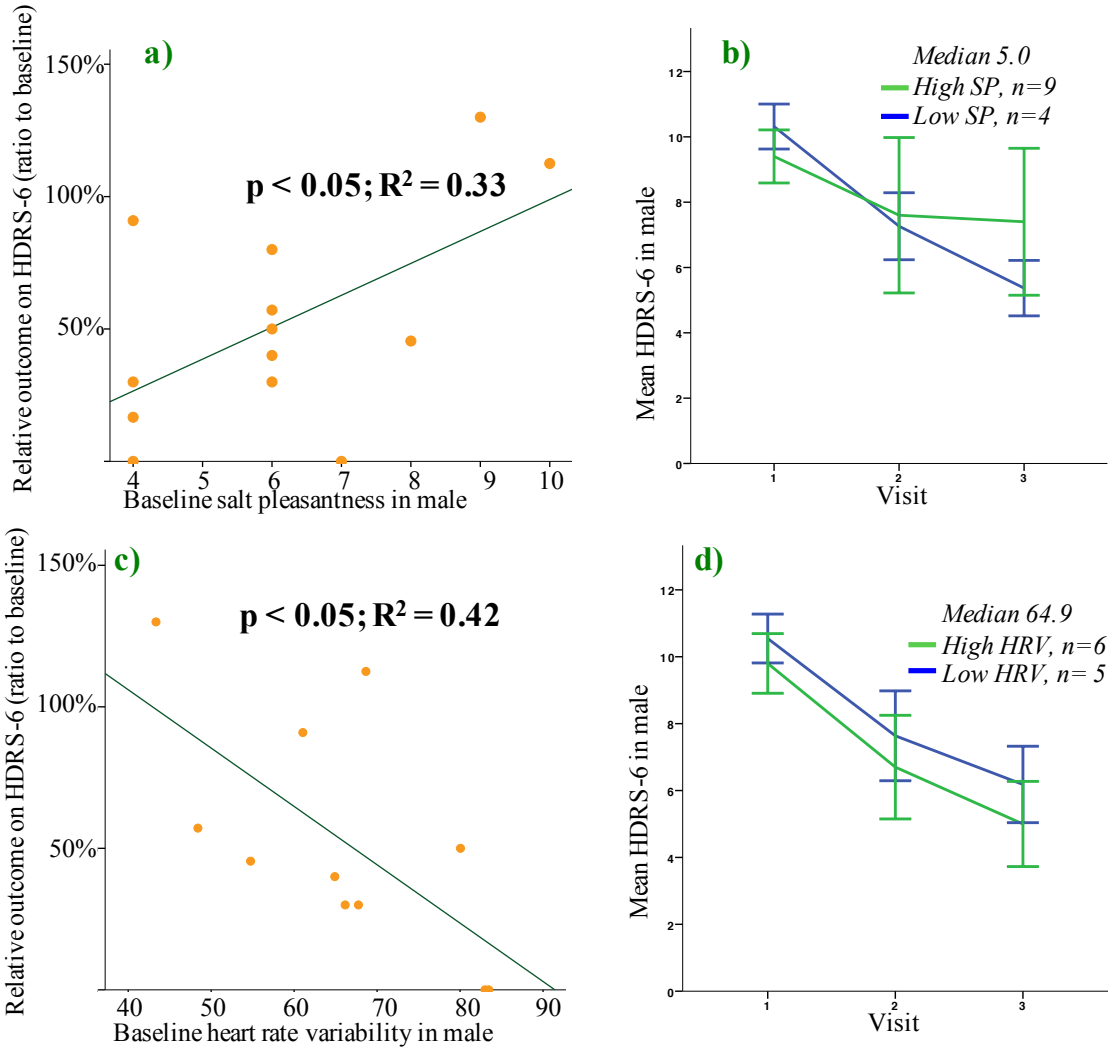
In the total population baseline HRV did not correlate with the relative change of HDRS-6 after six weeks (p = 0.40). *'[...] [However,] in male patients higher baseline HRV predicted a better response after 6 weeks ([...] for HDRS-21: p < 0.1; for HDRS-6: p < 0.05).'* (Büttner et al. 2015: 29) (Figure 8 c) and d)).

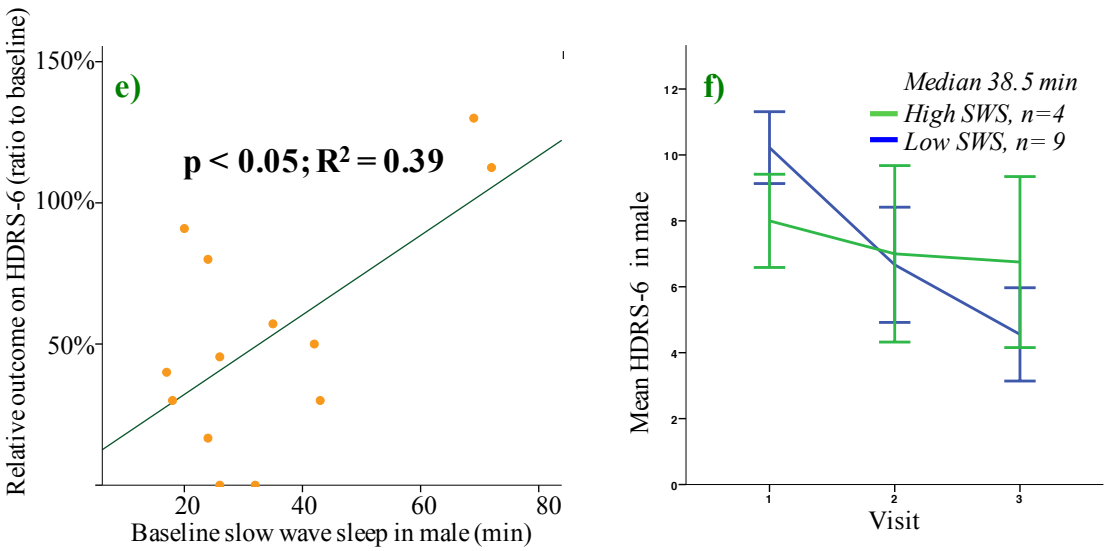
3.2.2.3. Slow wave sleep

In the total group there was no significant correlation of baseline SWS to the relative change of HDRS-6 (p = 0.18). *'[...] [However,] higher baseline SWS was predictive for a poorer response after 6 weeks [...] in male patients*' (Büttner et al. 2015: 29) on HDRS-21 (p < 0.05) (Figure 8 e) and f)).

Figure 8: Relationship between baseline markers of central MR activity (SP, HRV and SWS) and clinical outcome in male subjects

These figures show only selected results in male patients of the predictive value of baseline parameters for the clinical outcome and have been modified after Büttner and colleagues 2015. For a complete overview of the results of hypothesis one see Table 5. For explanations of the left and right column, x- and y-axis see Figure 5.
Figure a) and b) show salt pleasantness (SP) in male patients. Higher perceived SP at baseline (a correlate for salt appetite) is associated with unfavorable clinical outcome after six weeks (see also Figure 3).
Figure c) and d) show heart rate variability (HRV) in male patients. Lower baseline HRV in male patients was associated with unfavorable clinical outcome after six weeks.
Figure e) and f) on the next page show slow wave sleep (SWS) in male patients. Higher baseline SWS in male was associated with unfavorable clinical outcome after six weeks.





3.2.3. Peripheral MR markers

In the total group there was no significant correlation of baseline Mg^{2+} ($p = 0.98$), Na^+ ($p = 0.34$), K^+ ($p = 0.57$) or Na^+/K^+ ($p = 0.74$) levels to the relative change of HDRS-6. For male patients low baseline plasma Na^+ levels were by trend predictive for a poorer response after six weeks ($p = 0.050$) (Figure 9 a) and b)) (Büttner et al., 2015). This became significant on the proportional change of BDI in the total group ($p < 0.01$) and in male patients ($p < 0.02$) (Büttner et al., 2015).

In the total group and for female patients high baseline SBP predicted better improvement after six weeks ($p < 0.05$) (Figure 9 c) and d)) (Büttner et al., 2015).

In exploratory analysis: *'[...] [S]alivary aldosterone, when related to SBP completely separated responders (> 50% reduction of HDRS-21 score) from non-responders [...] [(Figure 10)].'* (Büttner et al. 2015: 29).

ANOVA with baseline SBP as dependent variable, response vs. non-response (as defined on HDRS-21) as inter-subject variable and baseline salivary aldosterone as covariate identified that responder had 26.87 mmHg significantly higher mean SBP than non-responder (142.50 ± 18.56 vs. 115.63 ± 14.34 mmHg, mean ± SD) at baseline ($p < 0.01$, $n = 18$).

Figure 9: Na^+ in male subjects and systolic blood pressure, prediction of clinical outcome

These figures show only selected results. For a complete overview of results for hypothesis one see Table 5. For explanations of the left and right column, x- and y-axis see Figure 5.
Figure a) shows that baseline Na^+ in male patients slightly missed significance as predictor of outcome after six weeks. Figure b) visualizes on a descriptive level that patients with lower Na^+ levels had higher absolute mean Hamilton Depression Rating Scale with 6 items (HDRS-6) scores over time.
Despite relatively high aldosterone levels at baseline (Figure 6 a)) lower systolic blood pressure (SBP) at baseline was predictive for clinical outcome after six weeks on HDRS-6 in the total group, i.e. male and female patients (figure c)). This was also seen on a descriptive level in figure d).

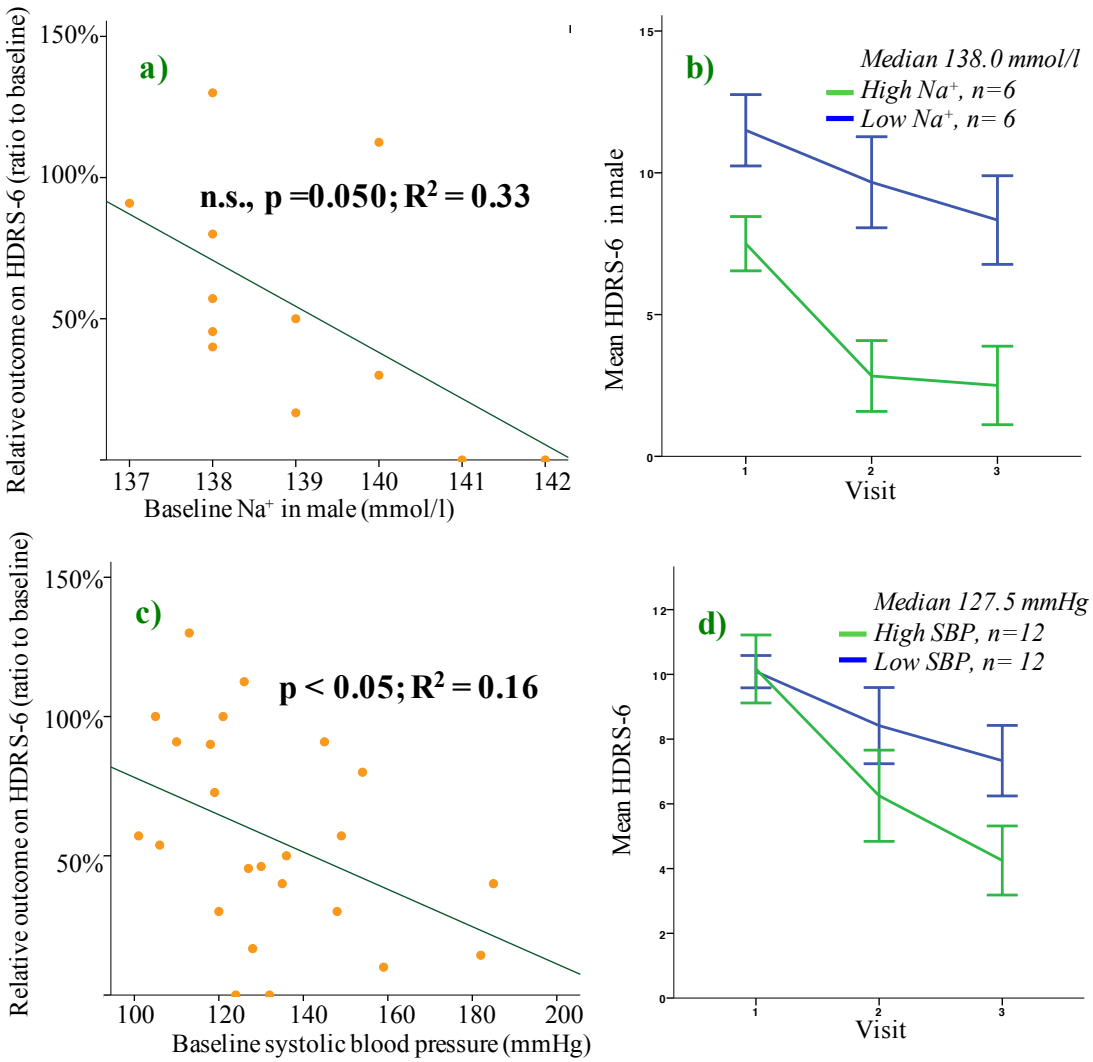
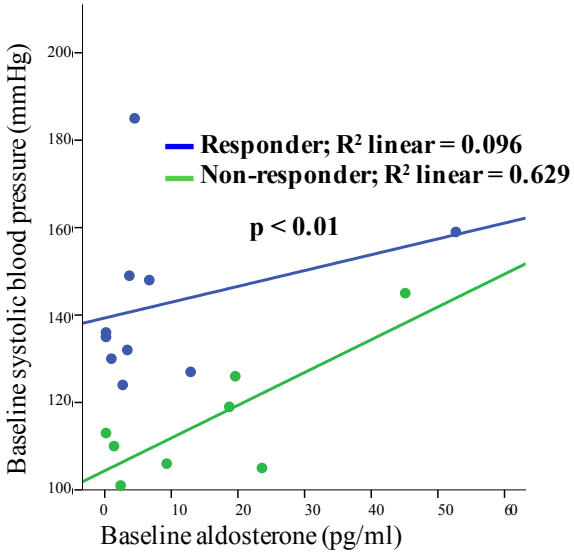


Figure 10: Salivary aldosterone and systolic blood pressure in responder vs. non-responder.

This figure has been modified after Büttner and colleagues 2015. '*Two clusters differentiated responders from non-responders. An overall lower systolic blood pressure [SBP] for a given aldosterone concentration supports the hypothesis of a reduced peripheral MR sensitivity.*' (Büttner et al. 2015: 32).
For this graph response was considered as a reduction of at least 50% of the baseline score and measured on the Hamilton Depression Rating Scale with 21 items.
Responder had significant higher SBP in relation to aldosterone than non-responder ($p < 0.01$, $n = 18$).



3.2.4. Influence of age, gender and RAAS modifying medication

ANOVA with gender as inter-subject variable and age as covariate showed that female patients had significantly higher baseline aldosterone values (19.7 ± 19.8 pg/ml, mean ± SD, n = 8) than male patients (5.1 ± 6.4 pg/ml, n = 10; $p < 0.05$). Accordingly, baseline potassium was significantly lower in female patients (3.6 ± 0.3 mmol/l, n = 11) than in male patients (3.9 ± 0.4 mmol/l, n = 12; $p < 0.05$). ANOVA revealed a further gender difference in SWS. Female patients had significantly more baseline SWS (56.3 ± 22.2 min, n = 11) than male patients (34.5 ± 18.0 min, n = 13; $p < 0.02$). Gender had no significant influence on any other baseline biomarker.

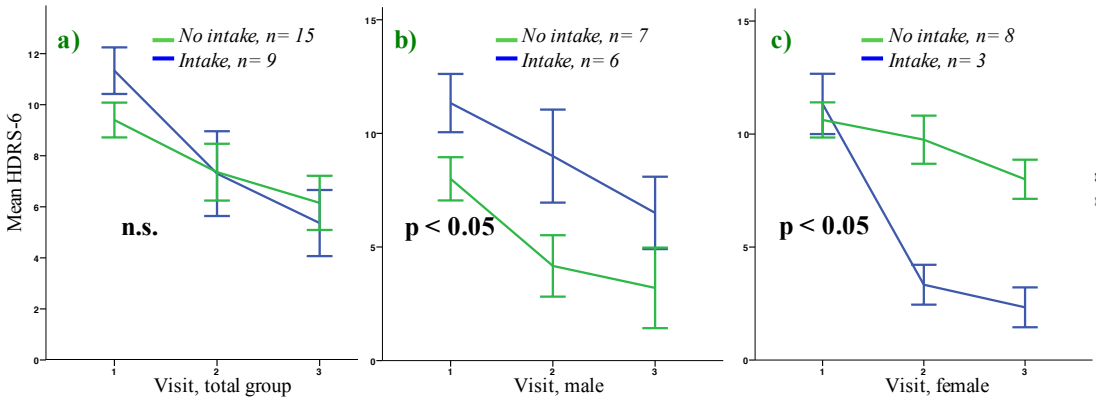
ANOVA with gender as inter-subject variable and age as covariate showed a significant influence of age on the baseline HRV (Büttner et al., 2015) ($p < 0.001$, $n = 21$), showing that younger patients had higher baseline HRV. Age had a significant influence on the baseline SBP ($p < 0.05$, $n = 24$), showing that older patients had

higher systolic baseline values. However, age had no significant influence on any other baseline biomarker.

RAAS modifying medication (i.e. ß-blockers, renin inhibitors, ACE inhibitors, ARBs) had been taken by about one third (37.5%) of the subjects at baseline (see Table 5). ANOVA with RAAS affecting baseline medication and gender as inter-subject variable and age as covariate showed that RAAS affecting medication (i.e. ß-blockers, renin inhibitors, ACE inhibitors, ARBs) had no impact on the course of depression on the HDRS-6 ($p = 0.51$, a)) but its influence became significant in the interaction with gender ($p < 0.05$). Male patients on this baseline medication ($n = 6$) only had a 36.2% reduction of depressive symptomatology after six weeks, whereas male patients not on this baseline medication ($n = 7$) had a 57.1% reduction of depressive symptomatology (b)). On the other hand, female patients on this medication at baseline ($n = 3$) had a 78.6% reduction of depressive symptomatology after six weeks, whereas female patients not on this medication ($n = 8$) only had a 23.7% reduction of depressive symptomatology after six weeks (c)). However, due to the small sample size these results have to be regarded with caution.

Figure 11: RAAS affecting medication and mean HDRS-6 over time

In this figure patients were separated on a descriptive level into those on RAAS affecting medication (i.e. ß-blockers, renin inhibitors, ACE inhibitors, ARBs) (blue) versus those not taking such medication (green). The x-axis represents visit one, two and three. The y-axis represents the score of the mean Hamilton Depression Rating Scale with 6 items (HDRS-6). Figure a) shows the total group of the study population. Figure b) shows only male patients. Male patients with intake of RAAS affecting medication had higher mean HDRS-6 scores. On the contrary, female patients in figure c) with intake of RAAS affecting medication had lower mean HDRS-6 score. However, there were only three female patients on RAAS affecting medication. Therefore, any interpretation has to be regarded with caution.
Error bars represent ± 1 standard errors of mean.



Importantly, the main outcome analysis of this work, the relationship between baseline aldosterone and relative improvement showed a positive trend as measured by HDRS-6 ($p < 0.08$, $n = 11$) and became significant on HDRS-21 ($p < 0.01$, $n = 11$)) in the group, which was free of RAAS modifying medication. In addition, the correlation between this clinical measure and aldo/cort ratio became significant ($p < 0.05$, $n = 11$) in this subgroup.

The relationship of SBP and response on HDRS-6 was not significant in patients free of RAAS affecting medication.

ANOVA with RAAS modifying medication as inter-subject variable showed, that patients with RAAS affecting medications had significantly higher blood pressure at baseline, most likely as an expression of the underlying hypertension ($p < 0.01$, $n = 24$) (Büttner et al., 2015) and had significantly lower baseline HRV ($p < 0.01$, $n = 21$). RAAS affecting medication had no influence on the other parameters.

'*For the other parameters, which were only significantly related to clinical outcome in males, a further selection of only male patients free of RAAS modifying agents led to groups too small for a meaningful analysis.*' (Büttner et al. 2015: 30).

Table 5: Results hypothesis one, baseline biomarker parameters and outcome

This table shows an overview of the results of hypothesis one and has been modified after Büttner and colleagues 2015. The parameters were grouped into three main groups: hormones, markers of central and peripheral MR activity. Green boxes represent positive correlations whereas red boxes represent negative correlations. Correlations for the total group were marked with: 'Tot' and trends ($p < 0.1$) with: '(Tot)'. Correlations for male patients were marked with: 'M' and trends with: '(M)'. Correlations for female patients were marked with: 'F' and trends with: '(F)'.
The proportional change of depressive symptomatology after six weeks relative to baseline was represented by the different rating scales: Hamilton Depression Rating Scale with 6 and 21 items (HDRS-6 and -21), Quick Inventory of Depressive Symptomatology, self-rating with 16 items (QIDS-SR-16) and Beck Depression Inventory (BDI). The biomarker parameters were: aldosterone, cortisol, ratio of aldosterone to cortisol (aldo/cort), salt taste intensity (STI), salt pleasantness (SP), heart rate variability (HRV), slow wave sleep (SWS), plasma concentrations of magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), ratio of sodium to potassium (Na^{+}/K^{+}) and systolic blood pressure (SBP).
Central MR activity markers (aldo/cort, SP and SWS) were positively related to an unfavorable course of depression. Low STI and HRV were negatively correlated with an unfavorable course of depression (Büttner et al., 2015).
In contrast, markers of a low peripheral MR activation (SBP and Na^{+}) appeared to be unfavorable for outcome (Büttner et al., 2015).

Scale	HDRS-6	HDRS-21	QIDS-SR-16	BDI
Hormones				
Aldosterone				Tot + F
Aldo/cort	(Tot) + (F)	Tot		
Cortisol				
Central MR activity markers				
SP	(Tot) + M	(Tot) + M		(Tot)
SWS	M	M		
STI		(Tot) + M		
HRV	M	(M)		(M)
Peripheral MR activity markers				
SBP	Tot + F	Tot + (F)		
Na+	(M)			Tot + M
Na+/K+				
K+				
Mg2+				

3.3. Results hypothesis two: Early response markers

This hypothesis examines the predictive value of the **early** change of biomarker parameters on the outcome. It examines predictive biomarker parameters that occur early in a depressive episode and therefore could explain a later treatment response. The early change of a biomarker parameter represents a ratio of its value at visit two divided by its baseline value (i.e. 100% corresponds to no change, 50% corresponds to a 50% reduction and 120% corresponds to a 20% increase of its baseline parameter activity, etc.). Table 6 gives an overview of the results of hypothesis two. For an easier understanding of the interpretation of the different figures see Figure 12.

Figure 12: Sketch for graph interpretation

This sketch was designed to facilitate the interpretation of the graphs of hypothesis two and three. It is drawn on the example of a positive linear correlation, described by the function $y(x) = b \cdot x + a$. In this example of a positive correlation the coefficient 'b' describes the gradient of the line and is considered > 0. The coefficient 'a' describes the axis intercept. A positive correlation is comparable with the green squares of Table 5, Table 6 and Table 7.

The x-axis in hypothesis two and three represents the proportional change of the selected biomarker parameter from baseline within two weeks (corresponding to the early change, hypothesis two) or within six weeks (corresponding to the late change, hypothesis three) after baseline, respectively. 100% corresponds to no change, 50% corresponds to a 50% reduction and 120% corresponds to a 20% increase of the baseline biomarker parameter value.

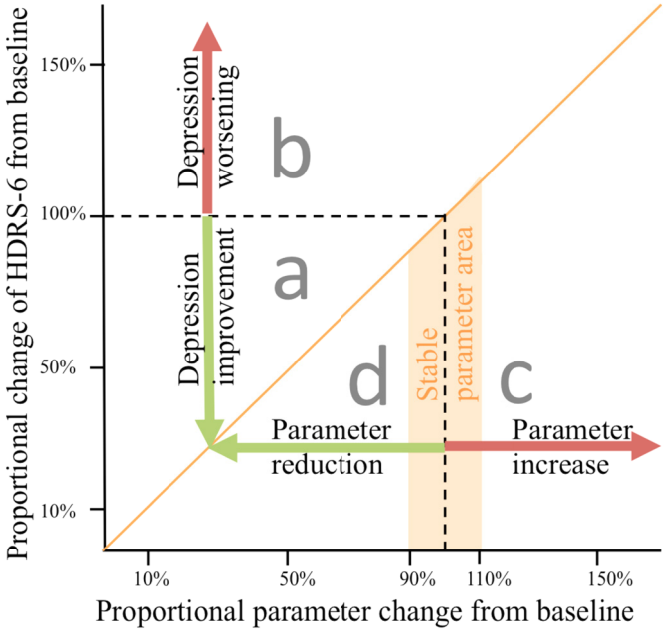
A stable biomarker parameter area was defined as a change within ± 10% compared to the baseline value. However, this differentiation was only done for the analysis of aldosterone change.

Similar to hypothesis one the y-axis represents the proportional change of the HDRS-6 score from baseline to six weeks after baseline (i.e. 100% corresponds to no change, 50% corresponds to a 50% reduction and 120% corresponds to a 20% worsening of its baseline HDRS score, etc.), i.e. it represents a relative value for antidepressant response.

For a summary of the different types of interpretations of a positive correlation the graph can be divided into four regions with two possible statements:

In region **(a)** and **(d)** a reduction of biomarker parameter activity is accompanied by an improvement of depression symptomatology.

In region **(b)** and **(c)** an increase of biomarker parameter activity is accompanied by a worsening of depression symptomatology.



3.3.1. Aldosterone and cortisol

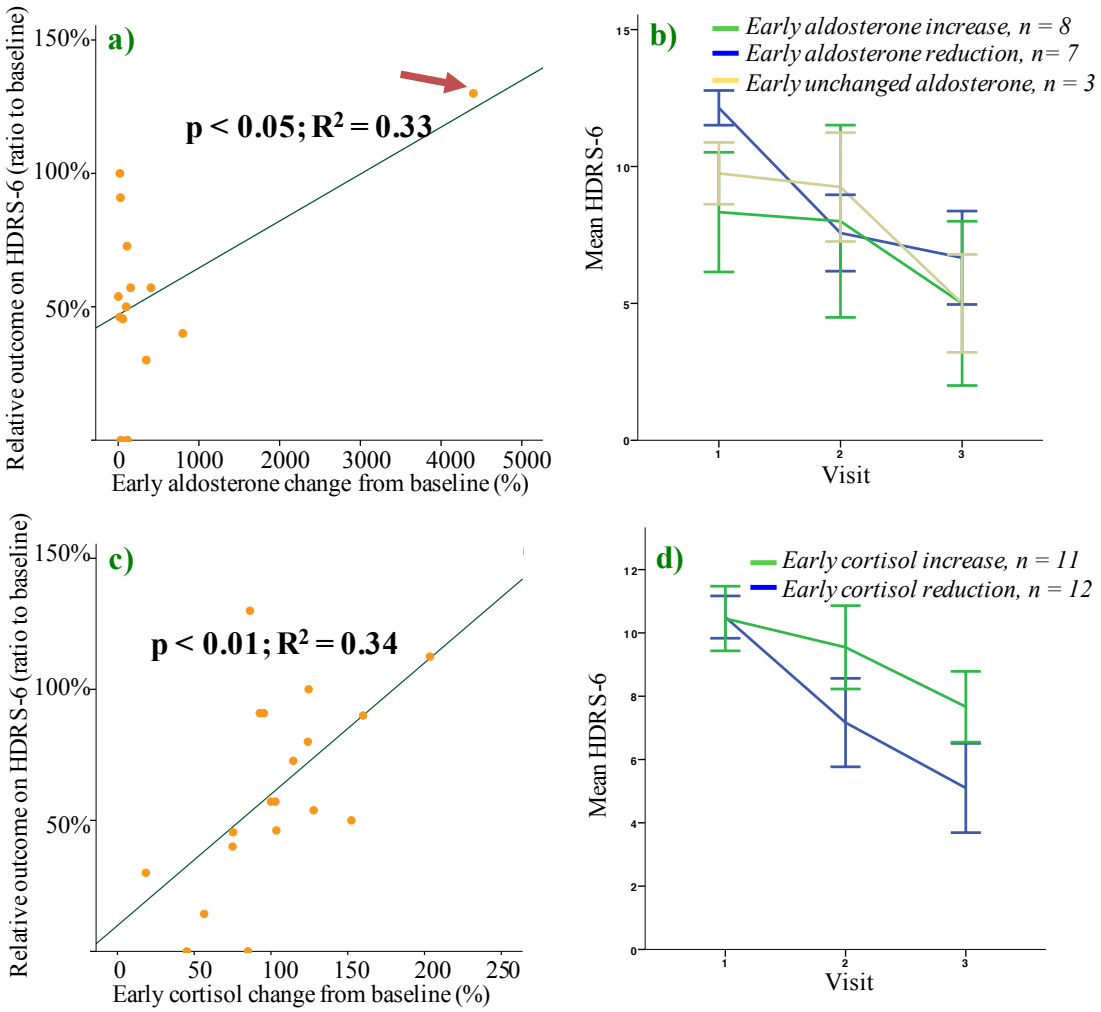
An early aldosterone reduction predicted a relative improvement after six weeks in the total group ($p < 0.05$) and in male patients ($p < 0.05$) (Figure 13 a) and b)). However, this correlation has to be regarded very cautiously as it is mainly based on one outlier (see red arrow in Figure 13 a)). This patient had a 4400% increase of his aldosterone concentration in comparison to baseline. If this outlier is taken out of the calculation the correlation was no longer existent ($p = 0.60$, $R^2 = 0.026$).

An early cortisol reduction in the total group ($p < 0.01$) (Figure 13 c) and d)) and in female patients ($p < 0.05$) was predictive for a favorable outcome.

In exploratory analysis a reduction of aldo/cort in female patients was by trend predictive for response on QIDS-SR-16 ($p < 0.08$).

Figure 13: The early change of aldosterone and cortisol and outcome

This figure shows only selected results of hypothesis two. For explanation of the axes and columns see Figure 5 and Figure 12.
Different to hypothesis one the x-axis here represents an early proportional change of the biomarker parameter from baseline to two weeks from baseline.
The positive correlation of aldosterone to clinical outcome was mainly based on one outlier (see the red arrow in (a)). This patient had a 4400% increase of his baseline aldosterone value at visit two.
A separation into three subgroups (reduction, unchanged and increase) was only performed for aldosterone (b).
Figures d) shows a separation of patients with early reduced parameter activity (i.e. below 100% of its baseline value; blue) and early increased parameter activity (i.e. these were patients with parameter values above 100%; green). If there was no change of parameter activity (i.e. exactly 100% of its baseline value) for the split for descriptive analysis this patient was assigned to the reduced parameter activity group.
In figure c) it can be seen that an early cortisol decrease was favorable for clinical outcome and unlike at baseline independent of aldosterone. This can be seen on a descriptive level in figure d).



3.3.2. Central MR markers

3.3.2.1. Salt taste intensity, salt pleasantness and heart rate variability

There was no relation between the early change of STI ($p = 0.58$), SP ($p = 0.64$) or HRV ($p = 0.51$) and the relative change of HDRS-6.

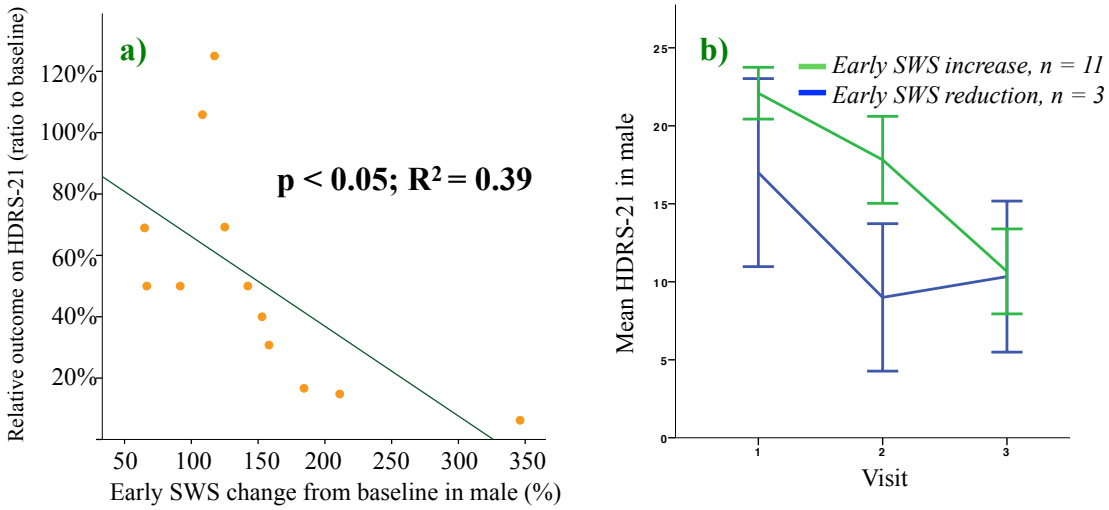
3.3.2.2. Slow wave sleep

An early SWS increase was by trend predictive for improvement after six weeks in the total group ($p < 0.06$) and in male patients ($p < 0.06$).

In exploratory analysis this became significant for male patients ($p < 0.05$) (Figure 14 a) and b)) on the relative change of HDRS-21 and the total group ($p < 0.05$) on the relative change of BDI.

Figure 14: Early slow wave sleep change in male subjects and outcome on HDRS-21

For explanation of the axes and columns see Figure 5, Figure 12 and Figure 13.
Figure a) shows the negative correlation of early slow wave sleep (SWS) change in male patients and outcome on the Hamilton Depression Rating Scale with 21 items (HDRS-21). Male patients with an early SWS increase within two weeks from baseline had a better improvement after six weeks vs. those with an early SWS decrease had a poorer improvement.
In figure b) male patients were divided on a descriptive level into early increased versus decreased SWS. Due to a small number (n = 3) in the reduction group this figure cannot be interpreted reliably.



3.3.3. Peripheral MR markers

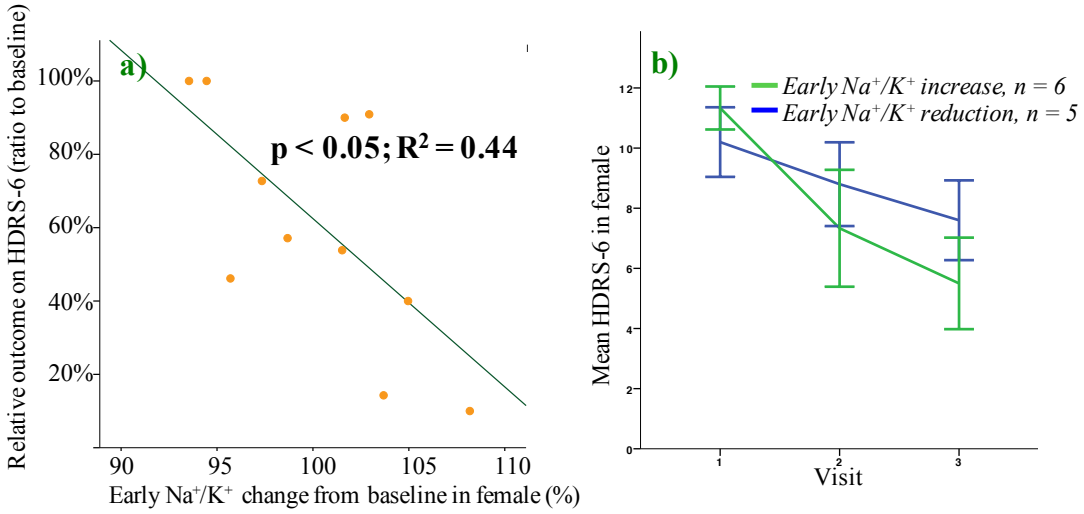
There was no relationship between the early change of Na^+ ($p = 0.66$) and Mg^{2+} ($p = 0.58$) and the primary clinical outcome measure. However, in exploratory analysis an early reduction of Na^+ was predictive for improvement in the total group on the proportional change of BDI ($p < 0.05$).

An early increase of Na^+/K^+ was predictive for improvement in female patients ($p < 0.05$) (Figure 15). An early K^+ increase was by trend predictive for improvement in female patients ($p < 0.06$).

There was no effect of an early SBP change on the primary outcome measure. However, in exploratory analysis an early reduction of SBP was by trend predictive for improvement on QIDS-SR-16 in the total group ($p < 0.1$).

Figure 15: The early change of Na^+/K^+ in female patients and outcome

For explanation of the axes and columns see Figure 5, Figure 12 and Figure 13.
In figure a) an early Na^+/K^+ reduction was unfavorable for clinical outcome in female patients, which was seen on a descriptive level in figure b).



3.3.4. Influence of age, gender and RAAS modifying medication

ANOVA with gender as inter-subject variable and age as covariate showed a significant gender difference concerning early SWS change ($p < 0.05$). Compared to baseline, female patients had an early SWS decrease (83.5 ± 34.6%, mean ± SD, n = 9), whereas male patients had an early SWS increase (147.4 ± 76.5%, n = 12).

ANOVA with gender as inter-subject variable and age as covariate identified a significant influence of age on the early changes of SWS ($p < 0.02$, $n = 21$), K^+ ($p < 0.05$, $n = 22$) and Na^+/K^+ ($p < 0.05$, $n = 22$). This ANOVA showed that younger patients had an early increase of SWS and plasma K^+, and an early decrease of Na^+/K^+. In contrast, older patients had an early decrease of SWS and K^+, and an increase of Na^+/K^+. Age had no significant influence on any other biomarker parameter of the early change.

The relationship between early cortisol change and relative improvement as measured by HDRS-6 stayed significant ($p < 0.05$, $n = 12$) in the group, which was free of RAAS modifying medication (i.e. ß-blockers, renin inhibitors, ACE inhibitors, ARBs = category one of somatic medication) at baseline. Additionally, in this subgroup the correlation between relative improvement and early SWS change became significant ($p < 0.02$, $n = 13$).
The outlier with a 4400% early aldosterone increase took RAAS modifying medication at all three visits. Therefore, the positive correlations for the early aldosterone change and outcome that occurred in the subgroup of patients taking RAAS affecting medication are not reliably analyzable.
ANOVA with baseline RAAS modifying medication as inter-subject variable showed that patients on RAAS modifying medication at baseline had by trend an early aldosterone increase ($p < 0.08$, n (total) = 14), a significant early cortisol decrease ($p < 0.05$, n (total) = 19), and a significant early increase of aldo/cort ($p < 0.05$, n (total) = 11) and HRV ($p < 0.01$, n (total) = 21). Whereas patients not on this medication tended to have early decreasing aldosterone values, early increasing cortisol values and an early decrease of the aldo/cort ratio and HRV compared to baseline. However, the trend of an early aldosterone change in patients on RAAS modifying medication could be influenced by the outlier with a 4400% early aldosterone increase.

Additionally, to the influences seen for the baseline medication ANOVA with RAAS modifying medication at visit two as inter-subject variable showed that patients on this medication had by trend an early K^+ decrease ($p < 0.06$, n (total) = 22) and an early Na^+/K^+ increase ($p < 0.09$, n (total) = 22).
Whereas oppositely, patients not on this medication had by trend an early K^+ increase, and by trend an early Na^+/K^+ decrease.

Table 6: Results hypothesis two, early change of biomarker parameters and outcome

This table gives an overview of potential early response markers. In hypothesis two the early change of biomarker parameters, i.e. their change within the first two weeks, was set into relation to clinical outcome of depressive symptomatology after six weeks. Parameters are grouped into three main groups: early hormonal, central and peripheral MR activity change.
For the interpretation of hypothesis two it is very important to keep in mind that hypothesis two looks at dynamic changes of parameter activities. Therefore, its interpretation is different to hypothesis one.
A reduction of cortisol (and aldosterone) was favorable for clinical improvement after six weeks. The relation of early aldosterone change to outcome should be interpreted very cautiously because the correlation was mainly based on one outlier and excluded. Therefore, a cortisol decrease was independent of aldosterone predictive for favorable outcome, i.e. therapy response. An increase of slow wave sleep (SWS) was by trend favorable for improvement.
The pattern of peripheral MR activation showed that a reduction of systolic blood pressure (SBP) was by trend associated with better outcome on the self-rating scale QIDS-SR-16. In female patients a Na^+/K^+ increase even in the presence of decreasing cortisol (and aldosterone) was more favorable for outcome. However, this is not in line with the observation that K^+ increase, which was seen by trend in female patients was associated with better outcome.
For explanation of abbreviations and colors see Table 5.

Scale	HDRS-6	HDRS-21	QIDS-SR-16	BDI
Early hormonal change				
Aldosterone	Tot + M	Tot + M		
Aldo/cort			(F)	
Cortisol	Tot + F	Tot + (M)		
Early central MR activity change				
SP				
SWS	(Tot) + (M)	(Tot) + M		Tot + M
STI				
HRV				
Early peripheral MR activity change				
SBP			(Tot)	
Na+				Tot
Na+/K+	F			
K+	(F)			
Mg2+				

3.4. Results hypothesis three: Surrogate markers of depression response

This hypothesis examines the relationship of the **late** change of the biomarker parameters on the clinical outcome. The late change of a biomarker parameter represents a ratio of its value at visit three divided by its baseline value (i.e. 100% corresponds to no change, 50% corresponds to a 50% reduction and 120% corresponds to a 20% increase of its baseline parameter activity, etc.). The late change of a biomarker parameter is regarded as a surrogate marker indicating if a neurobiological system has changed over time with regard to the clinical condition of a given patient. Table 7 gives an overview of the results of hypothesis three. For an easier understanding of the interpretation of the different figures see Figure 12 .

3.4.1. Aldosterone and cortisol

A late aldosterone reduction missed significance and showed by trend a relationship to clinical improvement in the total group ($p < 0.06$) and in male patients ($p < 0.08$). This correlation has to be regarded very cautiously as it is mainly based on one outlier (the same as in hypothesis two). This outlier had a 7100% late aldosterone increase in comparison to baseline. If this outlier is taken out of the calculation the correlation is again no longer existent ($p = 0.98$, $R^2 = 0.03$).

There was no relationship between the late change of either cortisol ($p = 0.79$) or aldo/cort ($p = 0.52$) and the proportional change of depressive symptomatology after six weeks.

3.4.2. Central MR markers

3.4.2.1. Salt taste intensity and salt pleasantness

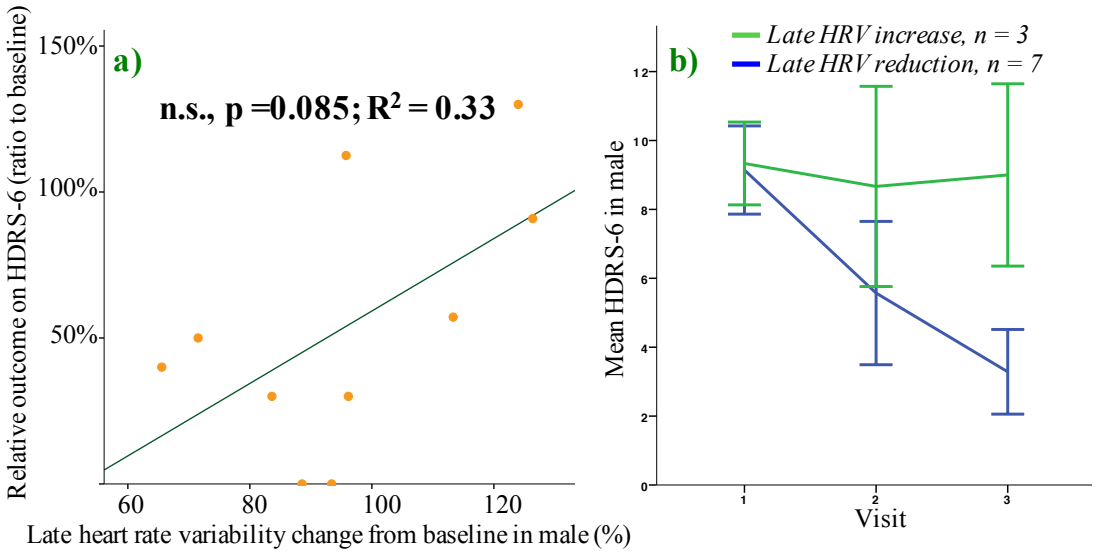
There was no relationship for the late change of either STI or SP on the primary outcome measure. In exploratory analysis, however, a late STI reduction as a marker of increased central MR activity was related to improvement in the total group ($p < 0.05$) and in female patients ($p < 0.05$) on the relative change of depressive symptomatology on BDI.

3.4.2.2. Heart rate variability

Only in male patients a late HRV reduction was by trend predictive for improvement ($p < 0.09$) (Figure 16 a) and b)). In exploratory analysis in male patients a late HRV reduction indicating improvement became significant for the relative change of depressive symptomatology on QIDS-SR-16 ($p < 0.02$) and on BDI ($p < 0.05$).

Figure 16: The late heart rate variability change in male subjects and outcome

For explanation of the axes and columns see Figure 5, Figure 12 and Figure 13.
The x-axis here represents a proportional change of the biomarker parameter from baseline to six weeks from baseline (i.e. 100% corresponds to no change, 50% corresponds to a 50% reduction and 120% corresponds to a 20% increase of its baseline biomarker parameter score, etc.).
Figure a) shows that male subjects with a late heart rate variability (HRV) reduction had by trend a better improvement.
On a descriptive level figure b) shows a separation of patients with late reduced parameter activity (i.e. below 100% of its baseline value; blue) and late increased parameter activity (i.e. above 100% of its baseline value; green). If there was no change of parameter activity (i.e. exactly 100% of its baseline value) for the split for descriptive analysis this patient was assigned to the reduced parameter activity group.



3.4.2.3. Slow wave sleep

There was no relationship between late SWS change and the primary clinical outcome measure ($p = 0.16$).

However, in exploratory analysis a late SWS increase was by trend related to improvement in the total group ($p < 0.08$) and in male patients ($p < 0.09$) on the proportional change of depressive symptomatology on HDRS-21. This trend was also present in the total group on the proportional change of depressive symptomatology on QIDS-SR-16 ($p < 0.08$).

3.4.3. Peripheral MR markers

There was no effect for the late change of the peripheral markers of MR activity, i.e. Na^+ ($p = 0.69$), K^+ ($p = 0.63$), Na^+/K^+ ($p = 0.55$), Mg^{2+} ($p = 0.50$) or SBP ($p = 0.48$), on relative clinical outcome after six weeks.

3.4.4. Influence of age, gender and RAAS modifying medication

ANOVA with gender as inter-subject variable and age as covariate showed that age had no significant influence on any of the parameters of the late change and revealed no significant gender differences concerning these parameters.

Patients on RAAS modifying medication (i.e. ß-blockers, renin inhibitors, ACE inhibitors, ARBs) at baseline had by trend a higher late aldosterone increase compared to patients not taking this medication ($p < 0.1$, n (total) = 16). However, this result could be biased by the outlier with a 7100% late aldosterone increase.
Unfortunately, after separating patients into subgroups of intake versus no intake of RAAS modifying baseline medication the positive trend for late HRV change in relation to outcome was lost ($p = 0.37$, $n = 7$ for intake, versus $p = 0.17$, $n = 13$ for no intake). In the group free of RAAS modifying medication the trend of late SWS change, as seen above, on the exploratory outcome measures HDRS-21 and QIDS-SR-16 was then also seen on the primary clinical outcome measure HDRS-6 ($p < 0.06$, $n = 15$).
Separating into intake versus no intake at visit two and three revealed no further relationships for the late parameter change and clinical outcome.
The outlier with a 7100% late aldosterone increase took RAAS modifying medication at all three visits. Therefore, the positive correlations for the late aldosterone change and outcome that occurred in the subgroup of patients taking RAAS affecting medication are not reliably analyzable.

ANOVA with RAAS modifying medication as inter-subject variable revealed no interactions between the RAAS modifying medication at visit two and three and the late change of biomarker parameters.

Table 7: Results hypothesis three, late change of biomarker parameter and outcome

This table gives an overview of the results for hypothesis three. In hypothesis three the late change of biomarker parameters, i.e. their change within six weeks, was set into relation to clinical outcome after six weeks. Similar to Table 5 and Table 6 the parameters were grouped into three main groups: late hormonal, central and peripheral MR activity change. For explanation of abbreviations and colors see Table 5.

For the interpretation of hypothesis three it is important to keep in mind that hypothesis three, like hypothesis two, looks at dynamic changes of parameter activities.

Similar to the results of hypothesis two the results for aldosterone should be interpreted cautiously because the correlation was mainly based on the same outlier as for hypothesis two and excluded.

In concern of the late change of SWS (and of aldosterone), the directions of the results of the early change of these markers were similar. A late increase of SWS correlated with a better improvement and thus potentially represented an increased central MR activity in responder.

A late HRV reduction in male patients was correlated to a better outcome on the self-ratings scales QIDS-SR-16 and BDI, a result in line with a reported effect of antidepressants. In the total group, a late STI reduction was associated with a better outcome on the BDI scale.

For peripheral markers of MR activation neither a late reduction nor an increase in their activity was associated with outcome.

Scale	HDRS-6	HDRS-21	QIDS-SR-16	BDI
Late hormonal change				
Aldosterone	(Tot) + (M)	(Tot) + (M)		
Aldo/cort				
Cortisol				
Late central MR activity change				
SP				
SWS		(Tot) + (M)	(Tot)	
STI				Tot + F
HRV	(M)		M	M
Late peripheral MR activity change				
SBP				
Na+				
Na+/K+				
K+				
Mg2+				

4. Discussion

The aim of this study was to assess a possible predictive value of aldosterone and functional MR dependent systems (Büttner et al., 2015) as well as their plasticity on treatment outcome in depression. The first hypothesis tested whether baseline MR activity is predictive for clinical outcome (Büttner et al., 2015) and therefore can be used as pre-treatment characterization of a patient (Murck et al., 2015).

In hypotheses two and three the presence of dynamic changes was examined in relation to outcome. The plastic entities in the context of this work were a reduction or an increase of MR functioning in the course of depression. In general, in a dynamic system higher and lower values of biomarker parameters at baseline, respectively, determine an evolution that is most likely bidirectional. For example: it is more likely for the biological systems of a given patient with a parameter value in the lower range to increase in the course of time than to further decrease. A further decrease of an already low parameter would cause a further much stronger derangement of the biological systems and is therefore unlikely.

The second hypothesis was whether an early reduction of MR activity is predictive for a favorable clinical outcome and therefore indicate if a given parameter can be used as a predictive marker for a later recovery. However, if there was an early change in a specific parameter this does not necessarily indicate that this change can also be seen later on because an early change could induce functional plasticity.

And the third hypothesis examined whether a late unchanged MR activity is a surrogate marker or surrogate endpoint for an unchanged clinical condition, i.e. therapy refractoriness. A surrogate marker is considered as a substitute for a clinical end point and is expected to go in parallel with clinical benefit, harm, or lack of benefit or harm (Biomarkers Definitions Working Group., 2001; Murck et al., 2015).

To verify these hypotheses a number of biomarkers associated with MR functionality were examined.

4.1. Summary of results

In summary, in this study a heterogeneous group of patients was present. This heterogeneity was seen for example in a wide range of participants' age. Patients were between 18 and 75 years old. The distribution of depressive subtypes especially of depression with atypical features (33.3%) corresponded to that seen in other studies

where around 15% to 29% of patients with a major depressive disorder displayed depression with atypical symptoms (Thase, 2006).
The response and remission rates were 50.0% and 33.3%, respectively. They were comparable to studies with SSRIs as standard antidepressant treatment and similar clinical ratings scales (Trivedi et al., 2006).

To get a more global perception for the MR functioning this summary presents the results of the primary clinical outcome measure (relative change of HDRS-6) as well as exploratory clinical outcome measures (for an overview see Table 5, Table 6 and Table 7). For the interpretation of the results it is important to keep in mind that co-linearity for the biomarker parameters was assumed, as each biomarker parameter was selected to represent the activity of MR function. Therefore, no correction for multiple testing was carried out.
Higher central MR activation at baseline was associated with a poorer outcome. A higher salivary aldosterone concentration and ratio of aldosterone to cortisol at baseline predicted a poorer outcome after six weeks. This was also seen for lower STI and higher SP at baseline, both signs of salt appetite. Only in male subjects low (abnormal) HRV and high SWS were associated with a poorer outcome (Büttner et al., 2015).
The pattern of parameters related to peripheral MR activation pointed to a MR dysfunction in the periphery, i.e. outside of the brain. This was reflected in low Na^+, and low SBP both of which were related to a poor outcome. The assumption of peripheral MR dysfunction arose, because despite the presence of relatively high aldosterone, which would normally lead to high plasma Na^+ and SBP, the opposite was observed (Büttner et al., 2015).
An early cortisol and possible aldosterone reduction, defined as a change within two weeks from baseline, predicted improvement. In responder this pointed to a reduction of the hormonal axis. However, the results for aldosterone have to be considered with caution because the correlation was mainly based on one outlier. Besides the hormones, only an early SWS increase, as a marker of central MR activation, was associated with improvement.
In the course of time, a pattern of an early increasing peripheral MR sensitivity was more favorable for outcome. With regard to an improvement of depression the MR activation led appropriately to a reduction of cortisol (and aldosterone). In addition,

MR activation at the kidney increased Na^+/K^+ plasma concentration. The MR was thus able to increase Na^+/K^+ despite decreasing cortisol (and aldosterone) values. And additionally, MR reacted appropriately to a decreasing cortisol with a decrease in SBP that was seen by trend on an exploratory level. However, an increase of peripheral MR sensitivity was not in line with the observation that by trend an early K^+ increase in female patients, and on an exploratory level an early Na^+ reduction in the total group were associated with a better outcome.

With regard to the late change of SWS (and of aldosterone), defined as a parameter change within six weeks from baseline, the results of the early change of these markers kept stable. However, again the results for aldosterone have to be treated with caution. A late SWS increase, i.e. a sign of central MR activation, was associated with a better outcome. In line with an increasing central MR activation were a late STI reduction (as a sign of increasing salt appetite), and only in male patients a late HRV reduction, which were associated with a better outcome on the BDI scale.

For peripheral markers of MR activation neither a late reduction nor an increase in their activity was significantly associated with outcome.

Taken together at baseline a central MR activation and a peripheral MR dysfunction can be identified in non-responder to antidepressant therapy, which is in accordance with the original hypothesis for the predictive value of baseline characteristics (hypothesis one). In the course of time an increasing central as well as peripheral MR activation were then identified in patients that responded to antidepressant therapy. This was not in line with the original assumption, which hypothesized a reduction of these markers. Therefore, based on the data of this work a hypothesis-generated modification presents a characterization of two groups, i.e. responder and non-responder, in the following paragraph.

Since there were very broad inclusion criteria and few exclusion criteria this study represents a broad clinical spectrum, however, with the small number of patients present in this study the results should not be generalized. Chapter 4.5. Limitations below offers a detailed discussion on the limitations of this study.

4.2. Differentiation of responder and non-responder

On the basis of the results obtained in the three hypotheses of this work it is helpful to describe a model that could differentiate responder and non-responder to standard antidepressant treatment.

A non-responder can be described as having either no response or even a worsening of depressive symptomatology after six weeks with standard antidepressant treatment. This description tries to summarize a pattern of therapy refractoriness at baseline for the whole study population independent of clinical subtype, which is then set into relation to a pattern that can be found in responder over the course of time.

In non-responder to standard antidepressant treatment a high central MR function and a peripheral MR dysfunction is present in the beginning of a depressive episode (i.e. at baseline).

High aldosterone and relatively low cortisol levels indicate a therapy refractory patient at this stage. Additionally, this patient has several signs of high central MR activity. These are a high amount of SWS, a low HRV and salt craving (i.e. high SP and low STI). It would be interesting to know if this patient additionally adds salt to his food (Goldstein and Leshem, 2014).

Despite the presence of relatively elevated aldosterone levels Na^+ and SBP are low as signs of peripheral MR dysfunction. Although their values are within the normal range, this condition could be summarized as a mild hyponatremic, hypovolemic extracellular dehydration. This could be the beginning of a vicious cycle, because low peripheral Na^+ leads to a further central MR increase. As a consequence of increased aldosterone levels, Mg^{2+} gets washed out of the body. A Mg^{2+} decrease does not predominantly take place in plasma but is mainly an intracerebral and intracellular process (not observed in this study). This may worsen the clinical depression (G. A. Eby et al., 2011).

In contrast, in the course of time in responder an increase of central as well as peripheral MR activity is present and associated with improvement.

In responder cortisol decreases. Signs of an increased central MR activity are: increasing SWS, decreasing HRV and a decrease of STI or increase of SP, respectively (both signs of increasing salt appetite).

In the periphery an increasing of peripheral MR activity is present. This can be identified by an increasing Na^+ and an increase in sodium appetite. Also a decreasing K^+ can be observed. Additionally, in the course of time SBP decreases in responder what could be due to regulatory factors such as the SNS or decreasing cortisol levels (Figure 17).
In responder a low central MR activity and a high peripheral MR activity is present in the beginning of a depressive episode (i.e. at baseline). Potentially due to effective antidepressant treatment these patients are able to increase their central as well as peripheral MR activity in the course of time and therefore show an improvement of affective symptoms. In refractory patients these changes appear not to occur.

The described set of markers could differentiate between responder and non-responder to standard antidepressant therapy. Of note, the markers described are easily accessible in clinical routine, as well as in clinical trials.

Having discussed the model for a differentiation of responder and non-responder, it is also essential to mention the limitations of this model. Firstly, it only describes one type of non-responder. There could be other modes of biological patterns in different subtypes of depression. Secondly, in the course of time only two entities, i.e. increase vs. decrease were examined. A third entity with unchanged parameter activity could be helpful to identify a rigid biological system. Thirdly, it is only possible to hypothesize, which parameter is the leading part of the change. With the present data this is impossible to tell, because a direct examination of the MR activity is to date not possible. Fourth, the pharmacological effect onto the examined parameters is unknown. For example, HRV is known to be influenced by antidepressant medication, which could be even after recovery not necessarily be comparable to healthy controls. So probably the medication in general but also different kinds of centrally active drugs stimulates the plasticity of a given system and therefore bias the interpretation. Finally, it has to be mentioned that for some parameters no effects could be observed especially concerning the early and late change, therefore limiting the evidence for the description of a development in the course of time.

Figure 17 gives an overview of the interconnectivity between peripheral and central MR functions and their interaction with aldosterone and cortisol.

4.3. MR activation and outcome

The question arises what could have caused the difference between an unfavorable central MR hyperactivity at baseline to a favorable increase of central MR activation over time? Potentially non-responder already had a high central MR activity at baseline and thus were not able to further increase their MR activity in the course of time. Whereas responder started with a low central MR activity and were able to increase their MR activity in the course of time, which then was favorable for outcome. Nevertheless, the question, what causes the difference from an unfavorable central MR hyperactivity (at baseline) to an increased favorable central MR activation over time, is an open question.

Aldosterone:

'The aldosterone concentration itself did not predict the clinical outcome of the current dataset […]. However, the lack of correlation is based on the presence of one outlier with high aldosterone levels, but a […] [favorable clinical outcome]. Taking this outlier into account the remaining data […] demonstrate a strong relationship between high aldosterone and a worse outcome ($R^2 = 0.29$, $p < 0.05$). […] [O]f note […], the subject in question was not part of the analysis of the effect of the aldosterone/cortisol ratio, as no cortisol concentration was determined.' (Büttner et al. 2015: 30).

The positive correlation between an early (hypothesis two) and late (hypothesis three) aldosterone increase and an unfavorable depression outcome are excluded from the discussion at this point, because they possibly were based on one outlier.

Ratio of aldosterone to cortisol

After defining a baseline ratio of aldosterone to cortisol, a low aldo/cort ratio was associated with a better response. Indicating that higher baseline aldosterone in relation to (lower) baseline cortisol values was a marker of poorer outcome after six weeks. In this context low cortisol values were only in relation to aldosterone a predictor for unfavorable outcome. This is other than expected, as most of the previously published data focused on high cortisol values as being unfavorable for outcome especially in melancholic depression. A condition that has been associated with a hyperactivity of the HPA axis in these patients (Gold and Chrousos, 2002).

Also patients with treatment resistant depression tend to have higher cortisol levels (Juruena et al., 2013). However, in these studies cortisol was not set in relation to aldosterone and possibly low cortisol values could be a sign for the presence of atypical depression (Gold and Chrousos, 2002; Lamers et al., 2012b; Murck et al., 2012).

As an influence on clinical outcome and the early and late change of aldo/cort was not provable this could indicate that for the plasticity over time aldosterone was no longer primary. Therefore, potentially indicating that the RAAS or other factors such as the SNS regulating the adrenal activity (Figure 17) could be especially important in the beginning of a depressive episode (Büttner et al., 2015).

However, a trend in female patients for the ratio of the early change of aldo/cort showed that stable or decreasing aldosterone values and at the same time increasing cortisol levels were associated with improvement. However, this effect was only seen on the exploratory scale of the QIDS-SR-16. For a discussion on diverging results between self- and clinician rating scales and differences in genders see Chapter 4.5.2. Clinical rating scales and Chapter 4.5.3. Age, gender and MR function further below. No such correlations were found for the late change of aldo/cort and clinical outcome (hypothesis three).

Cortisol:

For a response to everyday challenges an optimal adaption of cortisol levels regulated by the HPA axis is crucial. This concerns physical as well as psychological responsiveness as both have an impact on cortisol secretion (E. R. de Kloet et al., 1998).

A correlation between baseline cortisol and clinical outcome was not confirmed (hypothesis one). In the course of time an early cortisol decrease (without a clear change in aldosterone) led to an improvement after six weeks. The result of the early cortisol change therefore was in line with a variety of previously published data (J. Beck et al., 2015; Gold and Chrousos, 2002; Juruena et al., 2009; Lamers et al., 2012b). Improvement associated with an early cortisol decrease could be especially valid for patients with melancholic depression because 41.7% of the study population displayed symptoms of melancholic depression (see Table 2). At least in these patients a connection between higher cortisol levels and depression seems to exist (Lamers et al., 2012b).

In certain conditions cortisol could have the possibility to bind to the MR at neuroanatomical areas that are specific for aldosterone, for example when the enzyme 11ß-HSD_2 is saturated. A saturation of the 11ß-HSD_2 could be prevalent in a state of cortisol excess under stressful conditions. Therefore cortisol, though to a lower extent than aldosterone, could have an impact on electrolyte regulation and other central and peripheral markers of MR function.

It is not clear, whether cortisol itself or the MR activity is the leading factor for recovery in this context. One plausible physiological explanation could be that a reduction of negative feedback mechanisms due to increased central (prefrontal cortex, hippocampus) or peripheral (pituitary, outside the BBB) MR activity itself could have led to the early cortisol reduction, which was favorable for outcome. Consecutively not the concentration of cortisol and aldosterone could have been the important regulatory mechanisms, but their evoked action at the level of the MR.

SWS:

Sleep associated symptoms, like lack of concentration, lack of interest, fatigue or insomnia are among the most common residual symptoms in responder to antidepressant therapy and therefore affect patients' psychosocial functioning (Fava, 2006). The patterns for sleep disturbances in depression are heterogeneous (Murck et al., 2012). In melancholic depression a lack of sleep with early morning wakening can be described clinically. In electroencephalography low amounts of SWS, a short rapid eye movement sleep (REM) latency and a high REM density can be found (Antonijevic, 2008). Contrary to melancholic depression in atypical depression patients clinically show hypersomnia and probably increased SWS. It is suggested that the MR mediates SWS, whereas the GR mostly is involved in changes in REM sleep. However, nevertheless GR also moderately seem to influence SWS (Born et al., 1991). Due to the connection of MR and SWS this work focused on SWS as sleep parameter.

The results for the early and late change of SWS (hypothesis two and three) are at first sight counter intuitive compared to the result of hypothesis one. In hypothesis one higher baseline SWS predicted lesser improvement after six weeks in male patients. In hypothesis two and three an early and late SWS increase were then favorable for improvement. A finding similar to earlier studies showing that the amount of SWS was age and gender dependent and lower in depressed patients

compared to controls (Armitage et al., 2000; Riemann et al., 2001). However, in the present work no control group was present, therefore limiting the comparability.
As pointed out above (Chapter 1.4. Markers of MR function, page 16), increasing SWS is a sign of central MR activation. An explanation could be that in responder an increased MR activity led to a SWS increase that was favorable for outcome. Whereas in non-responder a lack of SWS increase or SWS decrease was present.
It is important to mention that the results for SWS were only found in the total group and in male patients. For a discussion on gender differences please see Chapter 4.5.3. Age, gender and MR function below.

HRV:

HRV was measured in this work by the RSA mediated RMSSD, which reflects the parasympathetic modulation of the organism. A high baseline HRV was associated with a better clinical outcome after six weeks. A result comparable to a study in patients with major depression and stable coronary heart disease, where low nighttime HRV was predictive for a poor treatment response (Carney et al., 2016).
However, no clear predictive value of early HRV change could be demonstrated.
A late HRV reduction in men, as a sign of central MR activation, was associated with clinical improvement. The late HRV reduction in men is in line with increased MR activation in responder in the course of time, which was also seen for SWS. Furthermore, it is known that antidepressant medications cause HRV reduction (Licht et al., 2008). As antidepressant medication becomes effective over time it may reduce HRV in the course of treatment. Therefore, a late HRV reduction that was associated with an improvement could be a sign that a given patient sufficiently responds to a prescribed antidepressant medication. Additionally, patients that improved under standard antidepressant therapy potentially started with a low central MR activity at baseline, which then increased in the course of therapy and thereby reduced HRV.
For a final evaluation of HRV in depression one would have to examine medication free depressive patients, which is however difficult due to ethical reasons. Chapter 4.5.4. Medication further elucidates the impact of medication.

Salt appetite:

If the peripheral Na^+ level is low, this should lead to a central and peripheral MR activation and aldosterone increase by activation of the NTS, the nucleus accumbens

and via the RAAS (Schweda, 2015; Shekhtman et al., 2007). This then potentially causes salt appetite that results in an underestimation of a normal salted meal (i.e. low evaluation of STI) and leads to an increased salt intake.

At baseline high SP and low STI (i.e. signs of salt appetite and central MR activation) were associated with worse clinical outcome. In the course of time, a late reduction of STI (i.e. a sign of increasing salt appetite and central MR activation) especially in female patients was associated with improvement of depressive symptomatology. It can be speculated that patients with higher baseline STI, which was associated with better improvement, had a sufficient peripheral MR function and therefore were able to reabsorb enough salt in the course of time, despite their lack of baseline salt appetite. These patients were able to restore their salt balance in the course of time (i.e. a late STI reduction), which then was a surrogate for improvement of depressive symptomatology in this group of patients.

Electrolytes:

Parameters of peripheral MR function were represented by the electrolytes and the SBP. Na^+ and K^+ are regulated via the MR primarily by aldosterone. In addition, high cortisol concentrations can lead to similar effects with an increasing 11ß-HSD_2 saturation.

The low baseline Na^+ in non-responder was attributed to a peripheral MR dysfunction. '*Animal data demonstrate that an increased dietary Na^+ leads to reduced anxiety and depression like behavior (Frazier et al., 2013; Krause et al., 2011), while Na^+ depletion leads to […] increased anxiety and anhedonia (Morris et al., 2006; 2010). In both situations these effects appear to be mediated via a change of RAAS activity. For an alternative explanation it should be considered that changes in electrolyte and in particular Na^+ distribution have been reported early as a possible causal factor in depression (Shaw et al., 1969) pointing to a potential higher intracellular Na^+ concentration in depression.'* (Büttner et al. 2015: 32, 33).

An early Na^+/K^+ increase was favorable for improvement in female patients, potentially as a sign of increased peripheral MR sensitivity. As the result was only significant for female patients, this could be especially valid for female patients. For an easier understanding of this results in the following explanation patients are divided into two groups:

Firstly, patients which had a poorer response after six weeks showed in comparison to baseline an ongoing peripheral MR dysfunction. The peripheral MR dysfunction was seen in an early Na^+/K^+ reduction even despite a cortisol increase, which under certain conditions can impact the MR.

Secondly, patients with a better response after six weeks had an increase of peripheral MR sensitivity. The increase of peripheral MR sensitivity resulted in increased Na^+ levels even in the presence of relatively low cortisol or aldosterone values.

However, in the total group on the exploratory BDI scale a Na^+ increase was associated with poorer outcome. This is inconsistent with the assumption of a peripheral MR activation. As described before, peripheral MR activation also leads to a reduction of aldosterone secretion. Due to missing data and outlier this could not be confirmed in this study. In addition, this inconsistency may point to a difference in electrolyte regulation between genders. A previously reported finding that depression and Na^+ intake are negatively correlated in female patients but not in men supports this assumption. As serum and dietary Na^+ are related, this could be possibly due to higher dietary Na^+ intake in men (Goldstein and Leshem, 2014). Nevertheless, lower Na^+ intake was associated with a significant risk of mental distress in Japanese men (Shimizu et al., 2015). Under stressful conditions individuals can be distinguished by their urinary excretion of Na^+ and K^+. Subjects classified as Na^+ excreters experienced more anger and tended to be more suspicious (Rollnik et al., 1995). The findings of this work are consistent with animal models of Na^+ depletion causing depression-like symptoms, which are reversed by Na^+ intake. Interestingly in the animal model a Na^+ depletion results in reduced HRV potentially leading to adverse cardiac effects. Despite Na^+ depletion K^+ levels were unchanged (Grippo et al., 2006; Morris et al., 2006).

A correlation between baseline Mg^{2+}, its early and late change, respectively, and clinical outcome could not be confirmed.

Systolic blood pressure:

The overall pattern of peripheral MR dysfunction and central MR overactivity at baseline seems counterintuitive. What could be a pathophysiological mechanism leading to such findings? The peripheral MR hypofunctioning seen in this patient collective with reduced Na^+ and SBP could lead to a stimulated aldosterone production, which as a consequence worsens depression and anxiety via its central

action and lead to a vicious cycle. *'Interestingly, salivary aldosterone, when related to SBP completely separated responders (> 50% reduction of HDRS-21 score) from non-responders in a way consistent with this hypothesis […] [(Figure 10)].'* (Büttner et al. 2015: 29).

Furthermore, compared to controls in depressive patients treated with antidepressants lower blood pressure was observed (Licht et al., 2009), which was independent of age and the use of antihypertensive or psychotropic agents in elderly patients (age > 64 years) (Lenoir et al., 2008). However, reported results are heterogeneous as increased blood pressure has been reported in severely depressed patients (Gold et al., 2005). Additionally to an increased blood pressure, Gold and colleagues identified in medication-free depressed patients significantly increased norepinephrine in the cerebrospinal fluid. Increased central norepinephrine levels could therefore explain the SBP increase via the stimulation of the SNS in severely depressed patients, a finding that the authors describe to be mainly relevant for patients with melancholic depression (Gold et al., 2005). By contrast, in patients with atypical depression a low blood pressure could be prevalent.

'[A] study on the relationship of blood pressure increase and clinical change of depression […] [is currently not available]. Effects of antidepressants on electrolytes have not systematically been studied with newer antidepressants, so that this question is open as well. Furthermore, there is some indication that antidepressants lead to a reduction of peripheral RAAS activity, i.e. lower activity of the angiotensin converting enzyme and/or lower aldosterone levels (Ahmed et al., 2011; Gard et al., 1999). Therefore, at least some of the effects, which are predictive in therapy response, could be epiphenomena, related to off-target characteristics of antidepressants.' (Büttner et al. 2015: 32).

The beneficial effects of the influence of RAAS changing medications at baseline in the current study may be counted as a support for the active involvement of the RAAS in the pathophysiology of treatment refractory depression. Chapter 4.5.4. Medication and Chapter 4.6. Outlook offer a more detailed discussion on this topic.

There was a trend on the exploratory QIDS-SR-16 scale, where an early SBP reduction was favorable for outcome. The trend was very weak and only found on an exploratory self-rating scale (see Chapter 4.5.2. Clinical rating scales for a discussion on self and clinician rating scales, page 83). If this phenomenon may be related to an aldosterone decrease this needs further study, as the dataset for aldosterone had

several missing data. Given that treatment responder to antidepressant medication appear to have intact peripheral MR function, an early SBP reduction appears nevertheless plausible.
Of note, the time of measurement could be crucial for the determination of SBP as blood pressure could vary throughout the day in depression (see Chapter 4.5.1. , page 82).

In conclusion patients that did not respond to antidepressant therapy had at baseline a high central and low peripheral MR activity. This was seen for markers of central MR activation in high salt appetite, low HRV and high SWS. In these patients, baseline Na^+ and SBP were low as markers of peripheral MR dysfunction.
In the course of time an increasing central as well as peripheral MR activity then became present in patients that responded to antidepressant therapy. This was seen for markers of central MR activation like increasing SWS, decreasing HRV and a STI reduction (as a sign of increasing salt appetite). Additionally, in the presence of decreasing cortisol (a sign of increased pituitary MR activity) and possibly aldosterone levels an Na^+/K^+ increase (i.e. increased reabsorption of Na^+) was identified as a sign of increased peripheral MR activation.

4.4. Synopsis

The aim of this study was to identify underlying biological mechanisms that could predict or serve as a surrogate for the course of depression. MR function was assessed by the selected parameters measured in this work. The following paragraph aims to set the above-discussed results into the context of different subtypes of depression and explain, what could be an underlying biological mechanism for the specific and differentiated involvement of MR function in affective disorders.

Independent evidence for an MR dysfunction in refractory patients comes from studies with the prednisolone suppression test. Prednisolone has similar abilities like cortisol in terms of binding the MR and GR. Prednisolone inhibited Na^+ excretion in adrenalectomized rats, an index of MR activation (Pariante et al., 2002). Normally depressed patients react with suppressed HPA axis activity to this prednisolone suppression test, patients non-responsive to treatment do not show this HPA axis suppression supporting a role of peripheral MR desensitization in patients with

treatment-resistant depression (Juruena et al., 2013; 2009; 2010). This pattern of peripheral MR dysfunction therefore seems to be specific for treatment refractoriness, as non-refractory depressed patients did not show this effect (E. A. Young et al., 2003). These studies then conclude that an abnormal MR function (as measured by an impaired response to prednisolone) could be considered as a stable biomarker of a poor clinical course and the lack of any response to treatment. This is an interesting aspect as the sensitivity of the MR appears to be a trait rather than a state marker, because acutely depressed and recovered patients show no difference in terms of MR sensitivity (Büttner et al., 2015; Juruena et al., 2010). This is in line with the findings of peripheral MR dysfunction seen at baseline and over time in more therapy refractory patients. *'[…] [Also] a reduced hippocampal MR expression in depressed patients post mortem independent of the depressive state at the time of death [supports this trait marker hypothesis] (Klok et al., 2011).'* (Büttner et al. 2015: 26).

The association between baseline parameters and outcome appears to be most consistent to predict clinical outcome, whereas early biomarker changes showed more variability. The use of MR related markers as surrogate markers is least reliable (hypothesis three). The latter is most likely due to a high variability in the neurobiology of mood disorder, as pointed out here from the perspective of MR function. The importance of early plasticity, however, has been supported in trials describing an early sustained response of antidepressant medication: the outcome of the early response in the first two weeks predicted the final improvement (Papakostas et al., 2005). In fact, if there was no therapeutic antidepressant effect within two weeks the chances for a later therapeutic antidepressant effect were less likely (Henkel et al., 2009; Stassen et al., 1999).

4.4.1. Subtypes of depression

Are the biomarkers identified in this study related to clinical characteristics of non-response to standard antidepressant therapy? To find an underlying biological pattern that corresponds with a psychiatric entity of depression as a possible correlate of MR function, patients were divided according to vegetative symptoms into three subtypes, which were based on the presence or absence of typical or atypical vegetative markers, respectively (Mannel et al., 2010).

'[…] [It was hypothesized] earlier (Murck et al., 2012) that patients with atypical features, accompanied by more SWS and lower cortisol levels, may represent a group with higher central MR activity. In fact, the combination of a higher baseline aldosterone/cortisol ratio (i.e. relatively lower cortisol levels) with higher [baseline] SWS, which characterizes the less responsive patients in the current study, resembles patients with atypical features. In line with […] [findings from Conn's Syndrome], patients with atypical features demonstrate higher levels of anxiety (Novick et al., 2005) and lesser treatment response to SSRIs (Stewart et al., 2010) [and a clear gender bias towards females]. Higher [baseline] central MR activity would be explained by higher plasma aldosterone concentrations. This pattern of atypical symptoms with higher aldosterone levels is confirmed by observations in patients with hyperaldosteronism, which demonstrate higher levels of anxiety and somatization (Apostolopoulou et al., 2013; Künzel, 2012), i.e. symptoms, which are part of the atypical depression spectrum (Murck, 2003). […] [The] classification [in this study] on the basis of QIDS self-rating assessments, however, did not provide a clear distinction in response in this relatively small sample.' (Büttner et al. 2015: 33, 34).

Therefore, a different biomarker pattern in different depression subtypes on the basis of a larger population and more stringent assessments of atypical symptoms cannot be ruled out with the data of this work.

'Psychic anxiety plays an important part in depressive symptomatology and is a predictor of non-response (Clayton et al., 1991; Fava et al., 2008).' (Büttner et al. 2015: 33). As described before the anxiety item no. 10 (psychic anxiety) of the HDRS-21 was associated to the biomarker parameters in a bivariate, pooled correlation analysis of all available data, i.e. from all three visits, with PCC (Büttner et al., 2015). In these patients higher anxiety was significantly correlated with lower STI ($p < 0.05$, $n = 82$) and higher SP ($p < 0.05$, $n = 82$), both signs of salt appetite. Anxious patients had significantly higher Mg^{2+} levels ($p < 0.01$, $n = 79$), lower SBP ($p < 0.01$, $n = 82$) and male patients by trend lower Na^{+} levels ($p < 0.06$, $n = 43$) (Büttner et al., 2015).

'The pattern of these changes may provide the biological basis for anxious depression. As […] [these parameters] are easily available markers in many studies, this hypothesis is testable immediately in already existing large databases.' (Büttner et al. 2015: 33).

4.4.2. Affective symptoms in Conn's Syndrome and Addison's disease

With the results of this dataset, it is clear that aldosterone is not the only causal factor of a depressive disease. However, it seems to play a very important part in the pathophysiology of depression, as it acts in a network of different functions (Figure 17). Aldosterone and the RAAS are probably more important in the beginning of a depressive episode as predictors for the treatment response. To further elucidate its functions it may be instructive to review somatic diseases associated with aldosterone, which show an overlap with depressive symptomatology.

Of upmost importance is the Conn's Syndrome. Jerome Conn described primary alderosteronism in 1955 (Conn, 1955). In Conn's Syndrome primary hyperaldosteronism can be caused by a solitary adenoma or adrenal hyperplasia. Patients with this syndrome have anxiety and depression-like symptoms (Sonino et al., 2011). Women are more affected by this dysregulation and had higher mean levels of depression (Apostolopoulou et al., 2013). The treatment options for Conn's Syndrome are medication with spironolactone, an MR antagonist, or adrenalectomy. In the animal model it has been shown that adrenalectomy has an anxiolytic effect (Briones-Aranda et al., 2009; Künzel, 2012).

The contrary of Conn's syndrome is Addison's disease. In Addison's disease chronic adrenal insufficiency causes hypocortisolism and hypoaldosteronism. Symptoms like low blood pressure, hyponatremia and hyperkalemia were also found in this study and show some overlap. Additionally, in Addison's disease syncope, hypoglycemia, convulsions and fever are found. On a psychological level these patients have increased levels of anxiety and fear as well as decreased performance efficiency (Warmuz-Stangierska et al., 2009). This is important because neuropsychological symptoms could be the first sign of Addison's disease (Abdel-Motleb, 2012).

Overall, there is precedence for aldosterone regulation as a causal factor for affective disorders.

4.4.3. P-glycoprotein

When aldosterone is increased in the periphery, the question arises whether it has the possibility to enter the brain? Elise and Celso Gomez-Sanchez answered this question very well (E. P. Gomez-Sanchez and C. E. Gomez-Sanchez, 2012). They point to p-glycoproteins (p-gp) that actively pump specific compounds out of the brain. Despite the lipophilicity of aldosterone and cortisol this is also the case for them.

Nevertheless, aldosterone has been found throughout the brain after radiolabeling (Geerling and Loewy, 2009). The aldosterone transport out of the brain seems to be significantly less than for cortisol, therefore, possibly reducing the up to 1000 fold higher concentration of cortisol in the plasma compared to aldosterone, although the brain aldosterone concentration is still two orders of magnitude less than levels of corticosterone (E. P. Gomez-Sanchez and C. E. Gomez-Sanchez, 2012).

4.4.4. Molecular pathogenesis of MR activity

In this study a differentiation between central and peripheral MR function has been made. Several mechanisms could be part in different regulatory states of MR function as well as the RAAS system. Therefore, the following question arises: *'Is the observed pattern of biomarkers and their relationship to clinical response biologically plausible?'* (Büttner et al. 2015: 34).

1. Genetic polymorphisms

In the RAAS the ACE converts angiotensin I into angiotensin II. *'Genetic studies demonstrated that polymorphisms of the ACE and the angiotensin receptor gene, which are linked to higher biological activity, are also linked to worse outcome with standard treatment [of depression] (Baghai et al., 2001; Bondy et al., 2005). Furthermore, a common polymorphism of the MR has been linked to stress induced cardiovascular and HPA axis reactivity (Derijk et al., 2006). A[nother] common MR polymorphism, which leads to less MR protein expression, is related to higher aldosterone concentrations, similar to […] findings [of this study at baseline], but also to a higher systolic blood pressure (van Leeuwen et al., 2010).'* (Büttner et al. 2015: 34).

Another study found that the cortisol awakening response (CAR) as a marker for HPA axis activity was modulated by the MR -2G/C single nucleotide polymorphism only in patients with depression that were using SSRIs, showing that patients with the MR -2G/C single nucleotide polymorphism had the highest CAR. In female SSRI users with the MR -2C/C genotype the CAR was entirely blunted (Klok et al., 2011). Therefore, the genotype could modulate the effectiveness of a specific antidepressant medication.

2. Epigenetics

'Besides genetic influences, epigenetic factors have to be taken into account. Early life conditions, including maternal deprivation, an intense stressor, suppresses hippocampal MR expression and function in rats (Vázquez et al., 1996).' (Büttner et al. 2015: 34).

Patients with early life stress have a suppression of the CAR after the administration of the MR agonist fludrocortisone and the GR agonist dexamethasone, whereas in patients without early life stress no such suppression was found for fludrocortisone. This points to a MR agonist sensitivity in patients with early life stress and an GR/MR imbalance that could be prevalent in depressed patients with early life stress (Werne Baes et al., 2013).

3. Environmental factors

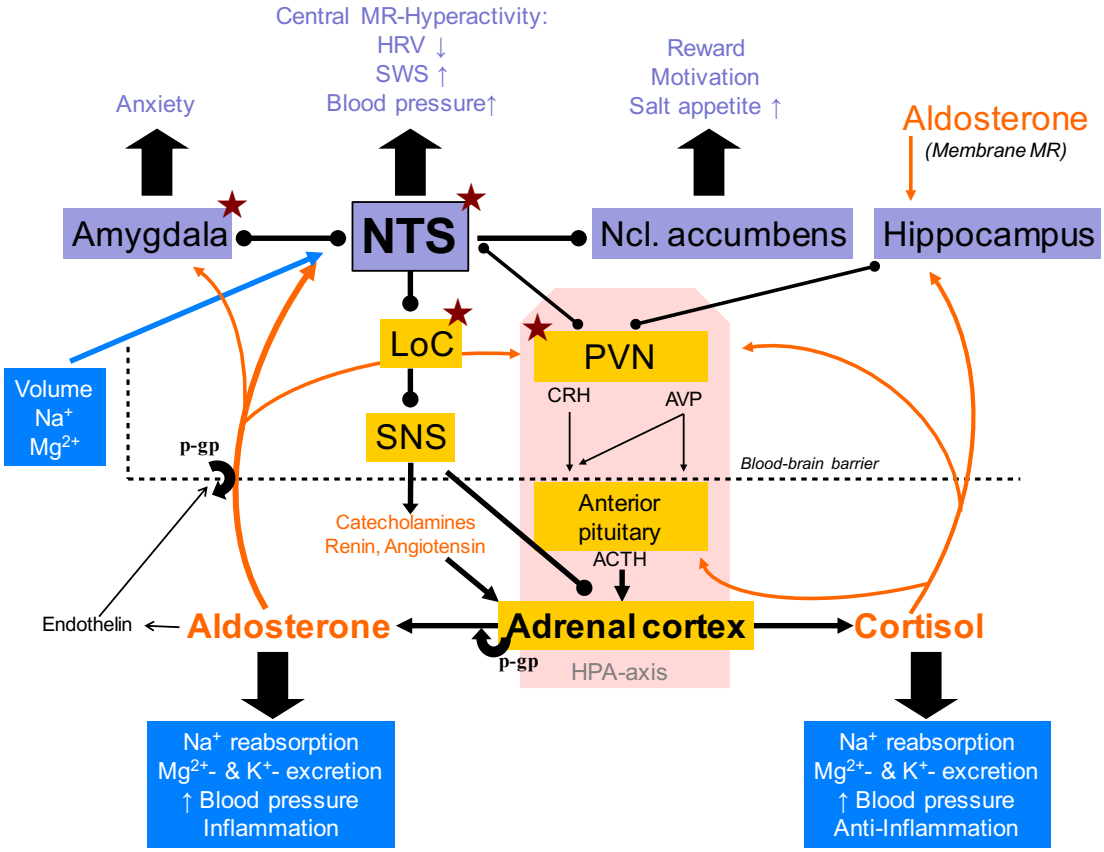
'[Additionally,] environmental challenges influence MR related behavior: it appears that sodium depletion in fetal development due to a variety of conditions determines sodium appetite in both animals and humans (Leshem, 2009; Leshem et al., 1993). The exact mechanism of these changes has to be further examined.' (Büttner et al. 2015: 34).

Taken together these three described MR mechanisms could lead to changes in MR function.

After having described different conditions that could lead to an alteration in MR function it is very important to mention that also gender and age can influence MR activity. For a discussion of gender and age see Chapter 4.5.3. Age, gender and MR function further below.

Figure 17: Overview of MR activity

This figure has been modified after Büttner and colleagues 2015. It gives an overview of aldosterone's action (endocrine influences are marked with arrows) in three interacting compartments: 1. In the periphery aldosterone regulates blood pressure, electrolytes, inflammation and increases endothelin production (Kohan et al., 2011) which potentially induces p-glycoprotein (p-gp). 2. Hippocampal membrane-bound MR has a relatively low affinity to cortisol, in comparison to intracellular MR, which allows aldosterone to successfully compete with cortisol. 3.Marked with red stars are actions of aldosterone on anatomical areas in the central nervous system, which contain 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD_2) and therefore allow aldosterone to bind to the classical intracellular MR. These areas are the nucleus of the solitary tract (NTS), the paraventricular nucleus of the hypothalamus (PVN) and the amygdala. These nuclei induce a wide spectrum of vegetative responses, are part of stress-regulation and the perception of emotion. Neuronal interconnections are displayed by 'drumsticks'. Further abbreviations are: adrenocorticotrophic hormone (ACTH), arginine vasopressin (AVP), corticotropin-releasing hormone (CRH), locus coeruleus (LoC), and sympathetic nervous system (SNS).



4.5. Limitations

4.5.1. Limitations of study conduct

a) The main methodological shortcoming of this study was the small number of patients. This did not allow a three-part division into patients with 'increasing', 'stable' or 'decreasing' parameters values, thus limiting the evaluation of hypothesis three.

b) To further emphasis the value of the used biomarker parameters the lack of a control group is another shortcoming of this study. This was especially relevant for the test on salt preference. Additionally, there was no control for other taste stimulants like for example a sugar solution, which could be another marker for unspecific taste sensitivity in major depressive disorder. However, in support of the role of saltiness, in animals it has been shown that Na^+-depleted rats prefer salt over other incentives (Smith et al., 1968), supporting the importance of salt as incentive.

c) *'Only hospitalized patients entered this study. This means that the population represented already some therapy refractoriness.'* (Büttner et al. 2015: 34).

d) To standardize the process of saliva samples collection for aldosterone and cortisol the waking up process was defined to be in a time period between 6:45 to 7:05 am. This was organized along the patients' awakening time, not specifically at a fixed point in time. The author woke the patient up in this period of time. However, if patients woke up earlier they were instructed to wait and stay in bed until the assessment was conducted. Nevertheless, strict adherence to the protocol cannot be guaranteed for all patients.

e) For the determination of SBP the time of measurement could be crucial, as blood pressure could vary throughout the day in depression. In this work SBP was measured in the morning after awakening at around 7 am right before the clinical self-rating and therefore could represent a state of rest, whereas a measurement in the evening could represent a state of stress. To avoid this time bias a 24-hour blood pressure profile could be a valuable tool.

f) The last clinical examination was performed 42 ± 7 days after the baseline examination. If patients were discharged earlier, due to faster recovery or other reasons, this last examination was moved up to the time of discharge, therefore could have caused some variability to the six weeks' values.

g) For diagnosis of depression no standardized diagnostic instrument, like the Structured Clinical Interview for DSM-IV, was used, but the clinical diagnosis procedure of experienced clinicians according to ICD-10 criteria for the diagnosis of depression.

h) The statistic concept of this work assumed linear relationships between biological and clinical parameters. In reality, however, the described systems could behave also in a non-linear way. Alternative assumptions of statistic distributions to a linear distribution were not analyzed.

i) Further limitations concerning the clinical ratings scales, the influence of age and gender onto the MR function as well as the medication are discussed below in detail.

'However, the consistency of the results across several markers [and hypotheses] provides support for an underlying pathophysiology and makes it less likely for the effects to be due to chance.' (Büttner et al. 2015: 34).

4.5.2. Clinical rating scales

'There is some inconsistency between the different clinical ratings: It appears that results differ between clinician rating scales such as the HDRS and self-rating scales such as the QIDS[-SR-16] and BDI scales. This could be in part because these two different types of scales were used at different [day]times and therefore represent different mood states of the patients. Clinician rating was performed in the evening [at around 4 pm] and self-rating in the morning after awakening [at around 8 am]. It is common that in depression mood and depression severity can change over the […] day. In this study the clinician rating scale was specified prior to study start as the primary one for analysis.' (Büttner et al. 2015: 34). To diminish the differences between self and clinician rating, these two should be performed at the same daytime.

The QIDS-SR-16 self-rating questionnaire was used to assign the patients to three depression subtypes based on vegetative symptoms (Chapter 2.8. Clinical outcome parameters, page 30). In this study sample there were no significant differences between subtypes. An explanation could be that in general during a depressive episode it is difficult for patients to evaluate their own vegetative symptoms. In particular, the self-assessment of patients' sleep could be biased. The self-assessment of sleep in the QIDS-SR-16 took place in the morning, after a short talk about the individual results of the sleep EEG. Therefore, the individual sleep evaluation could

be influenced by the patients' knowledge about the actual measured sleep parameters, which by observation differed in many cases from the self-perceptions.

To improve subtype evaluation clinicians should assign patients to the different subtypes based on vegetative symptoms with the help of a clinician rating scale such as the quick inventory of depressive symptomatology, clinician-rated with 16 items (QIDS-C-16).

4.5.3. Age, gender and MR function

It is important to state that age and gender have a considerable effect on MR and consecutively other parameters used in this study. Age in this study ranged from 18 to 75 years, representing a wide range.

The effect of age and gender on the MR has been summarized before by Ter Heegde and colleagues:

'One important factor affecting MR expression is age. Increased age is associated with decreased MR expression, resulting in a loss of HPA axis inhibition and, consequently, a chronic elevation of cortisol levels (Giordano et al., 2005; Heuser et al., 2000a; van Eekelen et al., 1991).' (Heegde et al. 2015: 96).

Therefore, for all related MR surrogate parameters age is an important confounder. Besides MR functioning, parasympathetic cardiac activity decreases with aging. This consecutively concerns HRV. In fact, a progressive HRV reduction has been observed until the age of 80. After this age a HRV increase starts again, which is then related to longer survival and predictive for longevity (Zulfiqar et al., 2010). Furthermore, HRV has been found to be significantly lower in healthy women compared to healthy men. These gender-related differences appear to decrease with age (Bonnemeier et al., 2003). Therefore especially in younger patients it is important to adjust HRV measurement for gender.

Concerning the parasympathetic activity, it is also important to keep comorbidities like diabetes mellitus in mind with a higher prevalence in older patients. Diabetes mellitus has a wide impact on autonomic nervous system function and therefore influences HRV (Xhyheri et al., 2012). The same is valid for SBP, where hypertension is more prevalent in older patients. The effect of age on HRV and SBP could also be confirmed in this study.

Furthermore, age was found to have a significant influence on the early SWS change (hypothesis two). The very well-known SWS decline with age could be confirmed in

this study. Age and gender dependence of sleep is very well documented. SWS is mostly constant in individuals but can vary widely across individuals (Mokhlesi et al., 2012; Riemann et al., 2001; Tan et al., 2001). It also seems that gender differences in terms of SWS are larger in depressed patients than in control. Men with major depressive disorder have the most disturbed SWS compared to healthy men, women and depressed women (Armitage et al., 2000). This could be one reason why in this study SWS showed only in male patients significant associations and trends towards treatment outcome, respectively.

Several of the following reasons can lead to substantial gender differences. One reason why there were more significant results for male than for female patients could be due to the female hormone cycle. Estrogen and progesterone regulate the female menstrual cycle. In the second half of the menstrual cycle progesterone, a steroid hormone like cortisol and aldosterone, is produced by the corpus luteum. Progesterone influences the HPA axis and therefore aldosterone (Carey et al., 1995). It is known that progesterone binds to the MR with nearly the same affinity as aldosterone or cortisol (Quinkler et al., 2002). Each part of the RAAS seems to be gender dependent and when dysregulated leading to anxiety especially amongst women (Apostolopoulou et al., 2013; Komukai et al., 2010).

Besides the influence of progesterone on the MR, it has been shown that women in the luteal phase of the menstrual cycle had higher aldosterone concentrations compared to women in the follicular phase and men (Hlavacova et al., 2013). Additionally, estradiol is important, as it primes the action of progesterone onto the MR (Barrett Mueller et al., 2014; Castrén et al., 1995; B. B. Turner, 1990).

In this study there was no control for the menstrual cycle. In this work gender was used as a stratifying factor leading to a separate evaluation of both genders. A correction would be especially necessary for the second half of the female cycle where progesterone, an MR influencing steroid, is predominant in female physiology. For further studies this could be improved by the assessment of the menstrual cycle.

4.5.4. Medication

'Patients [in this study sample] were treated mainly with SSRIs and mirtazapine, i.e. compounds with a monoaminergic mechanism of action [(see Table 4)].' (Büttner et

al. 2015: 34). Restrictions of co-medication were kept to a limit, but specific influences were anticipated.

Of note, aldosterone, MR activity and MR expression displays an interaction with antidepressants. For example chronic administration of fluoxetine or imipramine in rats leads to an increase in hippocampal MR (Brady et al., 1992; 1991). Furthermore, it has been shown that the intake of sertraline and escitalopram significantly increases aldosterone and reduces aldosterone to renin ratio in depressed men (Ahmed et al., 2011). Therefore, some of the taken antidepressants could already influence aldosterone and the MR in antidepressant treatment.

One important observation was that RAAS affecting medication (i.e. ß-blockers, renin inhibitors, ACE inhibitors, ARBs) had a significant influence on the clinical outcome (Chapter 3.1.3. Medication, page 39). However, gender was an important factor for this effect.

This highlights again a specific effect of gender in the regulation of MR. It was seen that the intake of RAAS affecting medication in female patients was associated with improvement. The opposite was the case in male patients. However, this observation has to be regarded with caution because in this work the numbers for a subdivision into gender and patients free of RAAS modifying medication were too small for a meaningful interpretation of the interaction of medication and clinical outcome. Therefore, a conclusive evaluation of RAAS affecting medication in depression has to be evaluated by larger clinical trials.

RAAS affecting medication had an impact on SBP. *'[…] [Due to the influence on SBP], the question arises whether the intake of this class of compounds and not higher blood pressure itself was related to a better clinical outcome. Due to the relatively small sample size this question cannot be answered conclusively.'* (Büttner et al. 2015: 30). In patients with hypertension and depression increased aldosterone concentrations have been found, compared to only depressed or only hypertensive individuals (Hafner et al., 2013). This emphasizes the involvement of RAAS in the pathogenesis of depression. In addition, antihypertensive medication like the MR antagonist spironolactone could be used as add-on treatment in this subset of depression, i.e. depression with hypertension. This is especially important in the context of aldosterone being a proinflammatory and fibrosis-inducing hormone explaining the link between cardiovascular diseases and depression.

Further, other parameters were influenced by RAAS affecting medication: HRV, aldo/cort and by trend aldosterone, cortisol, increasing K^+ and decreasing Na^+/K^+ outcome. However, correlations of parameters (baseline aldosterone, baseline aldo/cort, early cortisol and SWS change), in patients (male and female) free of RAAS modifying medication support a more generalizable interpretation, independent of the influence of this class of medication.

Additionally, there has been some evidence that nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) lead to increased plasma aldosterone concentration (Knights et al., 2006). Due to the frequent use and prescription of NSAIDs this medication could influence a depressive episode. However, in this work no statistical evaluation of NSAIDs has been carried out.

4.6. Outlook

Do the findings of this study imply further treatment options to antidepressant treatment?

In line with the association of central MR activity and affective symptoms drospirenone, a progestin with anti-mineralocorticoid and anti-androgenic activity, has been shown to ameliorate affective symptoms of premenstrual syndrome and the more severe premenstrual dysphoric disorder (Halbreich, 2004; Rapkin and Winer, 2008; Wang et al., 1995; Yonkers et al., 2005). The symptoms of premenstrual dysphoric disorder share clinical signs of atypical depression. Additionally, increased aldosterone levels have been observed in patients with premenstrual syndrome (Rosenfeld et al., 2008).

Whether pharmacologic treatments with spironolactone or another centrally active MR antagonist or compounds, which reduce aldosterone levels, can be used as add-on treatment in depression has to be further evaluated. These drugs may be effective only in a subset of patients, for example patients with central MR activation or hyperaldosteronism, possibly with atypical features or in patients with depression and hypertension. Nevertheless, further research is needed to improve individualized medicine in patients with depression.

Besides pharmacologic antidepressant treatment there could be non-pharmacologic ways to treat an initial relative hypotension as well as Na^+ levels in the lower normal range in patients with depression, two phenomena seen in this work. Modification of

one's lifestyle, like for example mild physical exercise, i.e. walking or swimming, constantly throughout the week, could be one treatment option. Physical exercise helps to improve response to treatment in depressed patients (Silveira et al., 2013) and balance cardiac autonomic function. In patients with unbalanced autonomic function caused by myocardial infarction physical exercise was a helpful therapeutic strategy to improve HRV. As discussed earlier also in depression autonomic nervous system dysfunction leads to a reduced HRV (Kemp et al., 2010) that could possibly be reversed or moderated with the help of physical exercise.
If the patient is not hypertensive increased salt and water intake, by adding extra salt to the meals and willfully drinking enough water every day, could be another additional naturalistic treatment options. Particularly amongst men adding salt could be a natural mechanisms of self-medication (Goldstein and Leshem, 2014).

Additionally to further treatment options, a detailed description of a depressed patient in the process of diagnosis and therapy monitoring could be very helpful. This work provided some biomarkers that could allow a better description on a biological basis for the monitoring of a patients' progress in antidepressant therapy and therefore could be a valuable tool for a personalized medicine.

4.7. Conclusion

In conclusion the progress in analytical techniques and electronic systems allowed the assessment of electrophysiological parameters with low expenditure and sufficient reliability. These methodologically independent markers of MR activity were able to predict treatment response to standard antidepressant treatment in hospitalized patients. Especially some of the used markers are particularly easy accessible (SBP, plasma electrolytes and salivary hormones) and exists already in other large databases. Therefore, parts of the hypotheses of this work can be easily tested. A newly developed test for salt preference describing baseline central MR function as well as its plasticity was associated with clinical outcome.
Taken together the results of this study as well as of independent studies suggest the MR functionality to be a trait rather than a state marker. This is in line with a peripheral MR dysfunction at baseline and over time in more therapy refractory patients in this work.

'[C]orrelates of higher baseline central MR activation are associated with poorer clinical improvement, particularly in men. This contrasted with the finding of a peripheral MR desensitization [at baseline] in more refractory patients. As one potential mechanism to consider, sodium loss on the basis of dysfunctional peripheral MR function and additional environmental factors may trigger increased aldosterone secretion and consequently worsen the clinical outcome.' (Büttner et al. 2015: 24).

Results for the parameter plasticity were heterogeneous. In the course of depression responder showed a cortisol decrease, an SWS increase, a HRV reduction and an increased salt appetite (i.e. STI decrease) as potential correlates of increased central MR function. In responder increased peripheral MR activity was identified by increasing Na^+. In contrast, non-responder appeared to demonstrate a reduction of central as well as peripheral MR activity.

'[Central MR hyperactivity at baseline, may] imply the possible therapeutic potential of centrally active MR antagonists for patients with treatment refractory depression, who demonstrate markers of central MR […] [hyperactivity].' (Büttner et al. 2015: 34).

Of note, important gender differences may exist in terms of MR function, as some of the reported findings were more consistent amongst male patients, whereas others amongst female patients (Büttner et al., 2015), thus making a correction for the female cycle necessary in future studies.

Finally, this and my previous work (Büttner et al., 2015) showed the practicability of the combination of easy to handle biological markers, to provide predictors of therapy refractoriness with standard therapy and is therefore an important step forward to an individualized therapy of depression.

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6. Appendix

6.1. Abbreviations

Abbreviations in alphabetical order:

11ß-HSD_2	11ß-Hydroxysteroid dehydrogenase type 2
ACE	Angiotensin-converting enzyme
ACTH	Adrenocorticotropic hormone
AD	Atypical depression
Aldo/cort	Ratio of aldosterone to cortisol
am	Ante meridiem, lt. before midday
ANOVA	Univariate analysis of variance
ARB	Angiotensin II receptor blockers
AVP	Arginine vasopressin
BBB	Blood-brain barrier
BDI	Beck Depression Inventory
BMI	Body mass index
Ca^{2+}	Calcium
CAR	Cortisol awakening response
CGI	Clinical Global Impression
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
DALY	Disability-adjusted life year
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders IV
ECG	Electrocardiography
EEG	Electroencephalography
ENaC	Epithelial Na^+ channel
Fp1	Left frontal polar EEG electrode
Fp2	Right frontal polar EEG electrode
Fpz	Middle frontal polar EEG electrode
GAF	Global Assessment of Functioning
GR	Glucocorticoid receptor
GTPase	Enzyme that hydrolyzes guanosine triphosphate
HDRS	Hamilton Depression Rating Scale
HPA axis	Hypothalamus-pituitary-adrenal axis
HRV	Heart rate variability
ICD-10	International Classification of Diseases Related Health Problems, 10^{th} revision
K^+	Potassium
LoC	Locus coeruleus
MAO	Monoamine oxidase
MD	Melancholic depression

Mg^{2+}	Magnesium
MR	Mineralocorticoid receptor
mRNA	Messenger ribonucleic acid
Na^{+}	Sodium
Na^{+}/K^{+}	Ratio of Na^{+} to K^{+}
NaCl	Sodium chloride
n.s.	Not significant
NSAIDs	Nonsteroidal anti-inflammatory drugs
NTS	Nucleus of the solitary tract
NV	Non-vegetative depression
p-gp	P-gylcoprotein
PA	Psychic anxiety
PCC	Pearson's correlation coefficient
pm	Post meridiem, lt. after midday
PVN	Paraventricular nucleus
QIDS-C-16	Quick Inventory of Depressive Symptomatology, clinician-rated with 16 items
QIDS-SR-16	Quick Inventory of Depressive Symptomatology, self-rating with 16 items
R^2	Coefficients of determination
RAAS	Renin-angiotensin-aldosterone system
REM	Rapid eye movement sleep
RMSSD	Root mean square of successive R-R-intervals
RSA	Respiratory sinus arrhythmia
SBP	Systolic blood pressure
SNRI	Selective norepinephrine reuptake inhibitor
SNS	Sympathetic nervous system
SP	Salt pleasantness
SPSS®	Statistical Package for the Social Sciences®
SSRI	Selective serotonin reuptake inhibitor
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
STI	Salt taste intensity
SWS	Slow wave sleep

6.2. Abbildungs- und Tabellenverzeichnis

Abbildungsverzeichnis:

Tabellenverzeichnis:

6.3. Bildnachweis

Figure 1: en.Wikipedia.org; http://en.wikipedia.org/wiki/Aldosterone#/media/File: Aldosterone-2D-skeletal.svg & http://en.wikipedia.org/wiki/Cortisol#/media/File: Cortisol2.svg

Figure 3: Photographs by Matthias Braunisch

Figure 4: Drawings by Matthias Braunisch modified after Zeo^{TM} user manual

6.4. Publications, abstracts and posters

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A) Publikationen:

1.

Title:

Target-based biomarker selection - Mineralocorticoid receptor-related biomarkers and treatment outcome in major depression

Matthias Büttner[1], Daniela Jezova[2], Brandon Greene[5], Carsten Konrad[1], Tilo Kircher[1], Harald Murck[1,3]

Journal of psychiatric research

Published: 2015

Abstract:

Aldosterone and mineralocorticoid receptor (MR) function have been related to depression. We examined central and peripheral parameters of MR function in order to characterize their relationship to clinical treatment outcome after six weeks in patients with acute depression. 30 patients with a diagnosis of major depression were examined 3 times over a 6-week period. Aldosterone and cortisol salvia samples were taken at 7:00 am before patients got out of bed. Easy to use e-devices were used to measure markers of central MR function, i.e. slow wave sleep (SWS) and heart rate variability (HRV). Salt taste intensity (STI) and salt pleasantness (SP) of a 0.9% salt solution were determined by a newly developed scale. In addition, systolic blood pressure (SBP) and plasma electrolytes were determined as markers for peripheral MR activity. The relationship between the levels of these biomarkers at baseline and

the change in clinical outcome parameters (Hamilton depression rating scale (HDRS)-21, anxiety, QIDS and BDI) after 6 weeks of treatment was investigated. A higher aldosterone/cortisol ratio (aldo/cort) ($n = 17$ due to missing values; $p < 0.05$) and lower SBP ($n = 24$; $p < 0.05$) at baseline predicted poor outcome, as measured with the HDRS, independent of gender. Only in male patients higher STI, lower SP, lower SWS (all $n = 13$) and higher HRV ($n = 11$) at baseline predicted good outcome $p < 0.05$). Likewise, in male patients low baseline sodium appears to be predictive for a poor outcome ($n = 12$; $p = 0.05$; based on HDRS-6). In conclusion, correlates of higher central MR activation are associated with poorer clinical improvement, particularly in men. This contrasts with the finding of a peripheral MR desensitization in more refractory patients. As one potential mechanism to consider, sodium loss on the basis of dysfunctional peripheral MR function and additional environmental factors may trigger increased aldosterone secretion and consequently worse outcome. These markers deserve further study as potential biological correlates for therapy refractory depression.

2.

Title:

Genetic, Molecular and Clinical Determinants for the Involvement of Aldosterone and Its Receptors in Major Depression

Harald Murck[1,3,4], Matthias Büttner[1], Tilo Kircher[1], Carsten Konrad[1]
Nephron Physiology
Published: 2014

Abstract:

Major depression (MDE) has metabolic and neuroendocrine correlates, which point to a biological overlap between MDE and cardiovascular diseases. Whereas the hypothalamic-pituitary-adrenocortical axis has long been recognized for its involvement in depression, the focus was mostly on cortisol/corticosterone, whereas aldosterone appears to be the 'forgotten' stress hormone. Part of the reason for this is that the receptors for aldosterone, the mineralocorticoid receptors (MR), were thought to be occupied by glucocorticoids in most parts of the brain. However, recently it turned out that aldosterone acts selectively in relevant mood-regulating brain areas, without competing with cortisol/corticosterone. These areas include the nucleus of the solitary tract (NTS), the amygdala and the paraventricular nucleus of the hypo-

thalamus. These regions are intimately involved in the close relationship between emotional and vegetative symptoms. Genetic analysis supports the role of aldosterone and of MR related pathways in the pathophysiology of depression. Functional markers for these pathways in animal models as well as in humans are available and allow an indirect assessment of NTS function. They include heart rate variability, baroreceptor reflex sensitivity, blood pressure, salt taste sensitivity and slow-wave sleep. MR activation in the periphery is related to electrolyte regulation. MR overactivity is a risk factor for diabetes mellitus and a trigger of inflammatory processes. These markers can be used not only to assist the development of new treatment compounds, but also for a personalized approach to treat patients with depression and related disorders by individual dose titration with an active medication, which targets this system.

B) Kongressbeiträge

I. Vorträge:

1. 12th World Congress of Biological Psychiatry, Athens, Greece, 2015

Lecture title:

Aldosterone and mineralocorticoid function as predictors of antidepressant response

Session title: Role of aldosterone and mineralocorticoid receptor function in emotion regulation

Matthias Büttner[1,*], Daniela Jezova[2], Carsten Konrad[1], Tilo Kircher[1], Harald Murck[1,3]

Abstract:

A dramatic change in our understanding of the neuroendocrinology of depression occurred during recent years. A renewed interest in the action of the mineralocorticoid receptor (MR) and its primary ligand aldosterone emerged. It became apparent that aldosterone sensitive sites exist in the CNS, which have relevant connections to sites of emotional regulation, i.e. the nucleus of the solitary tract (NTS), the paraventricular nucleus of the hypothalamus (PVN), the nucleus accumbens and the amygdala. Animal and human data support a causal involvement of aldosterone and the mineralocorticoid receptor (MR) in depression: Aldosterone is increased in several stress related models of depression. Aldosterone also mediates the motivation to acquire salt. Under conditions where animals display an appetite for salt but no saline

is available they display anhedonia. Blockade of the brain-renin-angiotensin-aldosterone system resolves both the appetite for salt and anhedonia. In patients with depression, increased salivary aldosterone is correlated with a more severe disease; the aldosterone/cortisol ratio at baseline predicts the outcome of antidepressant therapy in these patients. Further, salt appetite is high in patients with depression at baseline and shows a significant reduction in the course of antidepressant treatment. Similarly, low peripheral sodium-levels predict antidepressant non-response. The administration of the MR agonist fludrocortisones, which leads to a reduction of plasma aldosterone concentration, in patients with borderline personality disorder and in healthy controls leads to an increased emotional empathy without affecting cognitive empathy. Therefore, the involvement of aldosterone and MR function in stress related disorders demonstrates a close connection between basic homeostatic regulation and higher brain function.

2. 10th Annual Scientific Meeting of the International Society for CNS Clinical Trials and Methodology, New York, USA, 2014

Lecture title:

Target based biomarker selection – mineralocorticoid receptor related biomarkers and treatment outcome in depression

Session title: Taking Personalized Medicine Seriously: Biomarker Approaches in Phase IIb/III Studies in Major Depression and Schizophrenia

Harald Murck[1,3,*], Matthias Büttner[1], Daniela Jezova[2], Tilo Kircher[1], Carsten Konrad[1]

Abstract:

Introduction: Current research strategies for the identification of new targets often stress an unbiased theory-free approach, in particular in the context of the genetic-characterization. In contrast to that we would like to present the merit of a hypothesis-driven approach, focusing on mineralocorticoid receptor (MR) function. The involvement of the MR in the action of antidepressants is long known, more recently the involvement of aldosterone, as the specific ligand, has been described. We examined central and peripheral surrogate parameters of MR function for their relationship to outcome in patients with depression.

Methods: 33 hospitalized patients with major depression were examined 3 times during 6 weeks. Easy to handle and inexpensive methods for the characterization of MR related function were utilized: Morning aldosterone and cortisol salvia samples

were taken; parameters of MR function were slow wave sleep (SWS) and heart rate variability (HRV), systolic blood pressure (SBP) and plasma electrolytes. Patients evaluated perceived salt taste intensity (STI) and liking of a 0.9% salt solution. Depression severity was assessed with the Hamilton-Depression rating scale (HAMD-21).

Results: A higher ratio of salivary aldosterone/cortisol concentration at baseline was related to worse outcome. An early reduction (first two weeks) of aldosterone and cortisol predicted clinical improvement. STI increased in the course of treatment. Higher SBP at baseline predicted better clinical improvement. In male patients higher baseline Na^+, higher baseline HRV and higher STI predicted better improvement, whereas higher SWS predicted lesser improvement.

Conclusion: We demonstrated the feasibility to determine parameters of MR function in a routine clinical setting of hospitalized patients with depression. Our findings consistently point to a peripheral functional MR desensitization and a simultaneous increased central MR function as potential correlates of therapy refractoriness in hospitalized patients with depression, although gender differences may have to be considered.

3. 11th World Congress of Biological Psychiatry, Kyoto, Japan, 2013

Lecture title:

Role of mineralocorticoid-receptors in major depression

Session title: Aldosterone and mineralocorticoid-receptor function – an underlying cause of major depression or just an early marker?

Harald Murck[1,3,*], Matthias Büttner[1], Daniela Jezova[2]

Abstract:

Objective: Adrenocortical changes occur in major depression. In contrast to hypercortisolism, alterations of aldosterone are widely neglected. However, the expression of the mineralocorticoid receptor (MR), i.e. the receptor for aldosterone, is reduced in prefrontal areas of patients with depression; tricyclic antidepressants increase its expression; MR dysregulation explains several characteristics of melancholic depression and hyperaldosteronsism.

Methods: Concomitant measurement of SWS, salivary aldosterone and cortisol at awakening, heart rate variability, plasma renin at baseline, 2 and 6 weeks after baseline in patients with depression.

Results: The role of aldosterone was overshadowed by the belief that the MR is nearly always occupied by cortisol/corticosterone, which is an oversimplification. Under circumstances of a functional MR, a chronically increased aldosterone concentration leads to behavioral disturbances in both experimental animals and in patients with hyperaldosteronism on the basis of an andenoma. Here hyperaldosteronism is part of the cause in contrast of being just a biomarker. Functional MR overactivity leads to increased SWS, reduced HPA axis activity and other markers of MR overactivity, like arterial hypertension and increase in inflammatory activity. This form appears to have an overlap with atypical depression. This will be tested.

Conclusion: Both typical and atypical depression may be accompanied by hyperaldosteronism, but differ in MR activity. The ratio of aldosterone/cortisol or the ratio between aldosterone and slow wave sleep may lead to a clear biological distinction of these types and may determine treatment strategies.

II. Posterbeiträge:

1. 69th Annual Scientific Meeting of the Society of Biological Psychiatry, New York, USA, 2014

Title:

Central and peripheral mineralocorticoid receptor function and its impact on the course of depression

Matthias Büttner[1], Daniela Jezova[2], Carsten Konrad[1], Tilo Kircher[1], Harald Murck[1,3,*]

Abstract:

Background: Hyperaldosteronism and mineralocorticoid receptor (MR) dysfunction appear to play a role in depression. We examined central and peripheral surrogate parameters of MR function for their relationship to outcome in patients with depression.

Methods: 33 patients with major depression were examined 3 times during 6 weeks. Morning aldosterone and cortisol salvia samples were taken. Simplified devices were used for the measurement of slow wave sleep (SWS) and heart rate variability (HRV). Patients evaluated perceived salt taste intensity (STI) and liking of a 0.9% salt solution. Further systolic blood pressure (SBP), plasma electrolytes and depression severity (HAMD-6 & 21) were assed.

Results: Overall saliva-aldosterone and plasma Mg^{2+}concentrations were positively correlated with the HAMD-21 score ($p<0.05$). An early reduction (first two weeks) of aldosterone and cortisol did predict clinical improvement on HAMD-21 ($p<0.05$). STI increased in the course of treatment ($p<0.05$). In male patients higher baseline HRV and higher STI predicted better improvement ($p<0.05$), whereas higher SWS predicted lesser improvement ($p<0.05$). Overall sodium correlated by trend inversely with the HAMD-21 score ($p<0.06$). In men low baseline Na^+ ($p=0.050$) and low SBP in both genders predicted lesser improvement ($p<0.05$).

Conclusion: Lower aldosterone concentration or a decrease in its concentration corresponded to a better clinical symptom improvement. Low SBP, low plasma Na^+ and high plasma Mg^{2+} in the context of increased aldosterone concentrations points to a peripheral dysfunctional MR as a correlate of depression. This is in contrast to a simultaneous low STI, low HRV and high SWS as marker of central MR hyperactivity.

2. Kongress der deutschen Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde, Berlin, Deutschland, 2013

Title:

Aldosterone, salt craving and depression severity – a correlative analysis

Matthias Büttner[1,*], Daniela Jezova[2], Carsten Konrad[1], Tilo Kircher[1], Harald Murck[1,3]

Abstract:

Preliminary evidence points out to an involvement of aldosterone in the pathogenesis of depression (Murck et al., 2012). Aldosterone regulates salt appetite, blood pressure and has an impact on autonomic nervous system function. Therefore, these parameters could be functional markers of mineralocorticoid receptor (MR) function.

14 Patients were examined 3 times during their clinical stay, at baseline, 2 weeks and finally after 6 weeks after baseline or at discharge, respectively. For aldosterone and cortisol measurement saliva samples were taken immediately after awakening followed by measurement of heart rate variability (HRV). Patients then had to evaluate the taste of 0.9% NaCl in a cotton swab with two 11 point Likert scales: to determine 1. Subjective salt taste intensity (STI); 2. Pleasantness of taste (SP). Patients completed the Quick Inventory of Depressive Symptomatology (QIDS) for the determination of the severity of depression. For this preliminary analysis all time points were pooled for correlative analysis.

Aldosterone concentration correlated negative with STI ($p<0.05$) and correlated by trend with salt pleasantness (SP, $p<0.06$). STI and SP correlated negatively ($p<0.05$). QIDS score correlated positively with SP ($p<0.05$) and saliva aldosterone concentration ($p<0.05$). We could not confirm a correlation between aldosterone and HRV. Aldosterone correlated inversely (by trend) with plasma Na^+, pointing to the possibility of insufficient aldosterone mediated renal Na^+ reabsorption.

The results are in line with the hypothesis that aldosterone induces salt craving and depressive symptoms in patients with depression, however, the causes of the increased aldosterone levels have still to be determined.

3. 28. Symposium der Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie, München, Deutschland, 2013

Title:

Salivary aldosterone, salt craving and depression severity – a correlative analysis

Matthias Büttner[1,*], Daniela Jezova[2], Carsten Konrad[1], Tilo Kircher[1], Harald Murck[1,3]

Abstract:

Preliminary evidence points to an involvement of aldosterone in the pathogenesis of depression and regulates salt appetite (Murck et al., 2012). Salt appetite could be a functional marker of central mineralocorticoid receptor (MR) function.

14 Patents were examined 3 times during their clinical stay, at baseline, 2 weeks and finally after 6 weeks after baseline or at discharge, respectively. For aldosterone measurement salvia samples were taken immediately after awakening. Patients then had to evaluate the taste of 0.9% NaCl in a cotton swab with two 11 point Likert scales: to determine 1. subjective salt taste intensity (STI); 2. pleasantness of taste (SP). Patients completed the Quick Inventory of Depressive Symptomatology (QIDS) for the determination of the severity of depression. For this preliminary analysis all time points were pooled for correlative analysis.

Aldosterone concentration correlated negative with STI ($p<0.05$) and correlated by trend with SP. STI and SP correlated negatively ($p<0.05$). QIDS score correlated positively with SP ($p<0.05$) and saliva aldosterone concentration ($p<0.05$). No correlation was found between systolic blood pressure and any of these parameters.

The results are in line with the hypothesis that aldosterone induces salt craving and depressive symptoms in patients with depression.

4. Congress of the International Society of Psychoneuroendocrinology, Leiden, Netherlands, 2013

Title:

Aldosterone may induce salt craving and depressive symptoms – a correlative analysis

Harald Murck[1,3,*], Matthias Büttner[1], Daniela Jezova[2]

Abstract:

Preliminary evidence point to an involvement of aldosterone in the pathogenesis of depression. Aldosterone regulates salt appetite and blood pressure and has pro-inflammatory properties (Murck et al., Pharmacopsychiatry, 2012, 45: 83-95). Therefore, these parameters could be functional markers of mineralocorticoid receptor (MR) function.

14 Patents were examined 3 times during their clinical stay, at baseline, 2 weeks and finally after 6 weeks after baseline or at discharge, respectively. For aldosterone and cortisol measurement salvia samples were taken immediately after awakening. Patients then had to evaluate the taste of 0.9% NaCl in a cotton swab with two 11 point Likert scales: to determine 1. subjective salt taste intensity (STI); 2. pleasantness of taste (SP). Patients completed the Quick Inventory of Depressive Symptomatology (QIDS) for the determination of the severity of depression. For this preliminary analysis all time points were pooled for correlative analysis.

Aldosterone concentration correlated negative with STI ($p<0.05$) and correlated by trend with SP. STI and SP correlated negatively ($p<0.05$). QIDS score correlated positively with SP ($p<0.05$) and saliva aldosterone concentration ($p<0.05$). No correlation was found between systolic blood pressure and any of these parameters. Patients with increased C-reactive protein tended to have lower salivary cortisol ($P < 0.1$), but not aldosterone levels.

The results are in line with the hypothesis that aldosterone induces salt craving and depressive symptoms in patients with depression.

6.5. Studienprotokoll

Protokoll zum biomedizinischen Forschungsvorhaben „Veränderungen des Renin-Angiotensin-Aldosteron-Systems im Verlauf einer stationären Depressionsbehandlung“

Angenommen von der Ethikkommission des Fachbereichs Medizin der Philipps-Universität Marburg am 10.07.2012.

Verantwortlicher Leiter des Projektes vor Ort

PD Dr. med. Carsten Konrad, Facharzt für Neurologie und Facharzt für Psychiatrie und Psychotherapie, Oberarzt der Klinik für Psychiatrie und Psychotherapie des Universitätsklinikums Gießen und Marburg, Rudolf-Bultmann-Straße 8, 35039 Marburg, Email: Carsten.Konrad@med.uni-marburg.de, Tel.: 06421-58-65622, Fax: 06421-58-68939.

Sonstige Untersucher

PD Dr. med. Harald Murck, Facharzt für Psychiatrie und Psychotherapie, Senior Medical Director Neuroscience Medical and Scientific Services Convance Inc. - mit Lehrauftrag an der Philipps-Universität Marburg -, 206 Carnegie Center, Princeton, NJ, Email: haraldmurck@yahoo.de, Tel.: 201 294 1195 Fax: 609/9879262.

Matthias Büttner, cand. med., Doktorand der Klinik für Psychiatrie und Psychiotherapie am Fachbereich Medizin der Philipps-Universität Marburg, Email: mat.buettner@yahoo.de, Ketzerbach 51, 35037 Marburg, Tel.: 06421/2027543.

Studienrationale

Theoretischer Hintergrund:

Depressive Syndrome sind häufig, individuell stark beeinträchtigend und volkswirtschaftlich sehr teuer. Depressive Syndrome treten bei verschiedenen psychiatrischen Erkrankungen auf, insbesondere bei den affektiven Störungen wie z.B. der unipolaren Depression, der bipolaren Depression oder der Dysthymie. Die unipolare Depression ist die häufigste affektive Störung mit einer Lebenszeitprävalenz von weltweit etwa 16% (Kessler et al., 2003). In der Rangliste der Belastungen durch Erkrankungen (burden of disease) steht die unipolare Depression an erster Stelle aller Erkrankungen (WHO, 2008). Etwa jede 5. Depression verläuft chronisch (Kennedy et al., 2003). Von einer chronischen Depression spricht man ab einem Andauern der Symptomatik über einen Zeitraum von mindestens zwei Jahren (Keller et al., 1995; Gelenberg et al., 2006). Für die Behandlung depressiver Syndrome stehen effektive pharmakologische, psychotherapeutische und andere somatische Behandlungen zur Verfügung. Oftmals wirkt jedoch der zuerst gewählte Therapieansatz nicht. So konnte eine große amerikanische Studie (STAR-D) zeigen, dass bei einer Behandlung mit einem Selektiven Serotonin-Wiederaufnahmehemmer (SSRI) eine Vollremission lediglich bei ca. 30% aller Patienten erreicht wird (Trivedi et al., 2006). Gute Prädiktoren für die Auswahl einer Therapie fehlen, so dass es oftmals erforderlich ist die Therapieansätze nacheinander oder in Kombination einzusetzen. Wie weiter unten ausgeführt wird, ist ein wesentlicher Grund dafür die biologische Heterogenität depressiver Störungen. Parameter zur Differenzierung sind in einzelnen Studien erhoben worden, jedoch wegen der bislang technischen Komplexität auf geringe Fallzahlen und eine limitierte Anzahl von Parametern beschränkt. Dazu gehören insbesondere neuroendokrine Parameter, wie Kortisol-Bestimmungen, Entzündungsparameter, Schlaf-EEG-Parameter und Parameter des vegetativen Nervensystems, wie etwa die Herzratenvariabilität. Alle diese Parameter stehen in enger Verbindung mit der Regulation des Renin-Angiotensin-Aldosteron-Systems (RAAS), einem in diesem Zusammenhang bislang eher wenig untersuchten Stress-Hormon-System (Murck et al., 2012).

Durch den Fortschritt im Bereich der analytischen Technik und elektronischer Systeme ist es nun möglich, elektrophysiologische Parameter mit geringem Aufwand und ausreichender Reliabilität zu bestimmen und in Zusammenhang mit neuroendokrinen Parametern zu bringen. Genau dieser Ansatz ist Inhalt der vorliegenden Studie.

Zu testende Hypothesen:

Aus tierexperimentellen Untersuchungen kann folgende Arbeitshypothese generiert werden: Eine Stresssituation löst bei prädisponierten Menschen eine Reaktion aus, die es ermöglichen

soll, eine „Fight-oder-flight-Reaktion“ zu beginnen. Diese Reaktion geht mit einer Aktivierung des sympathischen Nervensystems und insbesondere mit einer vermehrten Aktivierung des RAAS und daraus folgend der Aldosteronkonzentration einher. Dieses führt zu einen Blutdruckanstieg, der dem Organismus die Verhaltensaktivierung ermöglicht. Dem folgt in kurzem Abstand eine Aktivierung der klassischen Hypothalamus-Hypophysen-Nebennieren-Achse mit einem Anstieg von Kortisol. Zum einen wird damit die Stressreaktion unterstützt. Wesentlich ist jedoch, dass Kortisol für viele Systeme ein Gegenspieler zu Aldosteron ist und z.B. akut stimmungssteigernd und antiinflammatorisch wirkt. Kortisol führt über Glukokortikoidrezeptoren in einer Rückkopplungsschleife zu einer Verminderung der ACTH-Freisetzung und damit zu einer Reduktion von sowohl Aldosteron als auch Kortisol. Dieses Rückkopplungssystem erscheint bei einigen Menschen zu wenig reaktiv zu sein, was zu einer verlängerten Stressreaktion und schließlich zu einer Depression führen kann.

Der neue Gesichtspunkt in dieser Hypothese ist, dass eine Aktivierung des Mineralokortikoidrezeptors (MR) depressiogen wirkt.

Folgende spezifische Hypothesen sollen dabei untersucht werden:

1. Eine funktionelle Überaktivität des Mineralokortikoidrezeptors (MR) verhindert eine Besserung der depressiven Symptomatik, insbesondere bei Behandlung mit SSRIs/SNRIs.
2. Das frühzeitige Auftreten (1-2 Wochen nach Therapiebeginn) von Markern einer verminderten MR-Aktivierung ist ein prädiktiver Marker für eine positive Response.
3. Eine unveränderte MR-Aktivierung deutet auf eine Therapierefraktärität hin.

Ziel der Studie:

Ziel der Studie ist die Charakterisierung von biologischen Parameters, die als Surrogatmarker für die Aktivität des Mineralokortikoidrezeptors angesehen werden können und deren Auswirkung auf die klinische Besserung bei Patienten mit einer Major Depression mit einer konventionellen antidepressiven Therapie.

Ethische Aspekte:

Heilversuch oder Wissensversuch

Es handelt sich bei unserer Studie um eine biomedizinische wissenschaftliche Untersuchung. Die Studie dient der Wissenschaft, die Teilnehmer haben keinen direkten persönlichen Nutzen. Es handelt sich nicht um eine Therapiestudie bzw. Interventionsstudie. Es sollen

Kenntnisse über unterschiedliche Grundlagenmuster des Körpers auf eine Therapie mit Antidepressiva gewonnen werden in Form der oben beschriebenen biologischen Parameter.

Durch die Untersuchung des Einflusses verschiedener Parameter soll Verständnis über den Zusammenhang verschiedener physiologischer Mechanismen erlangt werden. Dabei handelt es sich, wie oben beschrieben, um das RAAS im Zusammenspiel mit dem vegetativen Nervensystem, der Inflammation und der Zusammensetzung von Elektrolyten im Verlauf einer Depression. Die Untersuchung dieser Parameter dient der Fragestellung, ob sich Prädiktoren für das Therapieansprechen aus dem RAAS identifizieren lassen.

Dieses Wissen ist die Grundlage für das Verständnis pathologischer Prozesse, wie sie bei psychiatrischen Erkrankungen auftreten. Insofern dient die Untersuchung letztlich der Behandlung psychiatrischer Erkrankungen, da sie dem Kliniker in Zukunft mögliche biologische Parameter an die Hand geben sollen, die seine Entscheidungsfindung unterstützen können.

Gute klinische Praxis:
Die Studie wird unter Berücksichtigung der Richtlinien der *guten klinischen Praxis* (good clinical practice, GCP), entsprechend der Definitionen der *International Conference on Harmonisation* (ICH) und den ethischen Prinzipien der Deklaration von Helsinki durchgeführt.

Die Studie wird entsprechend der Vorgaben dieses Protokolls durchgeführt. Sowohl das Protokoll, als auch mögliche Ergänzungen und die Patientenaufklärung benötigen die Genehmigung durch die Ethikkommission der Philipps-Universität Marburg, bevor ein Patient eingeschlossen werden kann.

Verstöße gegen das Protokoll, die eine Gefahr für Patienten darstellen könnten, werden unverzüglich der Ethikkommission mitgeteilt.

Patientenaufklärung:
Die Untersucher legen dem Patienten eine Patientenaufklärung vor, die in nicht-technischer Sprache abgefasst ist. Dem Patienten wird ausreichend Zeit gegeben, sich über die Details der Studie zu informieren. Die Patientenaufklärung wird vom Patienten eigenhändig unterschrieben und datiert.

Wer informiert?

Die Aufklärung und das Einholen der schriftlichen Einverständniserklärung erfolgt durch einen ärztlichen Mitarbeiter der Klinik für Psychiatrie und Psychotherapie.

Allen Teilnehmern wird in dem Aufklärungsbogen zusätzlich die Möglichkeit angeboten, sich bei Rückfragen telefonisch oder per E-Mail an Herrn PD Dr. med. Carsten Konrad (Klinik für Psychiatrie und Psychotherapie der Universitätsklinik Marburg) zu wenden. Telefonnummer und Anschrift bzw. Emailadresse siehe Informationsblatt.

Studienablauf

Formal handelt es sich bei dem vorgelegten Projekt um eine reine Verlaufsbeobachtungsstudie. Therapeutische Interventionen außerhalb der gängigen klinischen Praxis sind nicht vorgesehen.

Studienplan

Bei Aufnahme wird eine Basischarakterisierung durch Diagnose, Erkrankungsdauer, Episodenanzahl, soziodemographischen Status etc. erhoben. Die Zustandsvariablen und die neurobiologisch-technischen Parameter, einschließlich Polysomnographie, Herzratenvariabilitäts-Analyse sowie spezifische Speichel- und Blutentnahmen finden zur Baseline-Untersuchung, kurz nach der Aufnahme, nach 1-2 Wochen nach Beginn der Studie sowie nach 6-7 Wochen nach dem Baseline-Zeitpunkt statt.

Dabei wird nach Möglichkeit versucht die Blutentnahme zur Bestimmung der Blutelektrolyte und der Entzündungsparameter mit den ohnehin im klinischen Alltag anfallenden Blutentnahmen zu synchronisieren, die normalerweise auf Station im zweiwöchigen Rhythmus stattfinden, so dass aufgrund der Studie nach Möglichkeit keine zusätzliche Belastung für die Patienten besteht. In besonderen Fällen können Ereignis-getriggerte Zwischenerfassungen eingeschoben werden, z.B. zu Beginn oder Ende einer besonderen Therapiemaßnahme, die sich aus dem naturalistischen Behandlungsverlauf ergeben. Am üblichen klinischen Behandlungsverlauf wird durch die Studie nichts verändert, nur die Verlaufsbeobachtung kommt hinzu.

Im Folgenden werden die zu erhebenden klinischen und biologischen Parameter aufgeführt und die Rationale für deren Verwendung dargestellt:

a) Klinisch-psychiatrische Symptomerfassung

Die Symptomschwere und Ausprägung des depressiven Syndroms soll durch Fremderfassung erhoben werden:

- Brief Psychiatric Rating Scale (BPRS; Overall and Gorham, 1962) besteht aus 18 Items, welche zu den fünf Skalen (1) Angst/Depression, (2) Anergie, (3) Denkstörung, (4) Aktivierung und (5) Feindseligkeit/Misstrauen gehören.
- Clinical Global Impression Scale (CGI; National Institute of Mental Health, 1985) dient der Beurteilung der Symptomschwere und -veränderung über die Zeit.
- Global Assessment of Functioning (GAF; Saß et al., 2003) bewertet das allgemeine Funktionsniveau in psychischen, sozialen und beruflichen Bereichen.
- Hamilton Depression Rating Scale (HRDS; Hamilton, 1960) mit 21 Items zur Erfassung depressiver Symptome. Zur Auswertung wird der HDRS-6 Subscore verwendet, dies wird weiter unten noch genauer ausgeführt (Bech, 2006; Lecrubier and Bech, 2007).

und durch Selbsterfassung:

- Quick Inventory of Depressive Symptomatology (QIDS-SR-16, Rush et al., 2003): Selbstbeurteilungsbogen mit 16 Items, der eine Einteilung in Depressionssubtypen erlaubt. Falls dieser vom Patienten nicht ausfüllbar ist, wird ersatzweise der Inventory of Depressive Symptomatology (IDS-C) durch den Untersucher als Fremderfassung durchgeführt.

  Die QIDS-SR-16 hat eine Schlüsselstellung für die Einteilung der Patienten in klinische Subgruppen, die unterschiedlich auf verschiedene Behandlungsverfahren ansprechen. In Analogie zu Murck et al., 2005, 2009 und in Anlehnung an Trivedi et al., 2008 werden Patienten in drei klinische Gruppen auf der Grundlage des QIDS-SR-16 eingeteilt:

  Im ersten Schritt wird eine Gruppe mit melancholischen Merkmalen definiert: Vorhandensein eines Scores von mindestens 2 von mindestens einem der QIDS-SR-16 Items: Item 3, Früherwachen; Item 6, verminderter Appetit; Item 8, Gewichtsabnahme.

  Im zweiten Schritt werden Patienten mit atypischen Merkmalen definiert: Vorhandensein eines Scores von mindestens 2 von mindestens einem der QIDS-SR-16 Items: Item 4, zu viel Schlaf; Item 7, vermehrter Appetit; Item 9, Gewichtszunahme.

  In einem dritten Schritt werden Patienten ohne diese vegetativen Merkmale zusammengefasst.
- Beck Depression Inventory (BDI) stellt eine vielfach verwendete Selbst-Beurteilungsskala dar.

Soziodemographische Variablen werden anhand eines Fragebogens erfasst. Die Eintragungen in ein zur klinischen Routine gehörendes Zustandsbarometer sollen ebenfalls wissenschaftlich ausgewertet werden und dienen der Kontrolle der Validität der Hauptzielparameter. Des Weiteren wird eine detaillierte Anamnese erhoben, bei der vor allem die Begleitmedikation (aufgeschlüsselt nach psychiatrischen, nicht-psychiatrischen und das RAAS beeinflussende

Medikamenten), Familienanamnese, Alkohol- und Nikotinanamnese sowie internistische und psychiatrische Diagnosen dokumentiert werden.

Parameter der klinisch-psychiatrischen Symptomerfassung, welche die Schwere und den Behandlungsverlauf determinieren können und eine biologische Grundlage haben, sind: Schweregrad, Dauer der Erkrankung sowie Dauer der depressiven Episode, Alter und Geschlecht. Spezifische klinische Merkmale (Vorhandensein von vegetativen Merkmalen 1. der melancholischen Depression, 2. der atypischen Depression oder 3. die Abwesenheit von vegetativen Parametern) werden mittels des QIDS-SR-16 definiert.

Daneben wird die Medikation der Patienten erfasst. Für die antidepressive Medikation werden differenziert: 1. SSRIs/SNRIs; 2. Mirtazapin; 3. MAO-Inhibitoren und Hypericumextrakte; 4. Atypische Neuroleptika; 5. Lithium. Für die nicht-psychiatrische Medikation werden Substanzen gesondert berücksichtigt, die einen Einfluss auf das RAAS haben: 1. ACE- und Renin-Inhibitoren, einschließlich Beta-Blocker; 2. MR-Antagonisten, wie etwa Spironolacton.

b) Neurobiologische Parameter des Renin-Angiotensin-Aldosteron-Systems

Es sollen funktionelle Systeme untersucht werden, die einen Bezug zur Regulation des RAAS haben. Dabei sollen Konstellationen von Parametern charakterisiert werden, die eine Nicht-Antwort (Non-Response) der antidepressiven Standardtherapie, insbesondere von SSRIs und SNRIs, vorhersagen können. Ziel ist es nun, frühzeitig Responder von Non-Respondern zu unterscheiden, um in Zukunft eine für den einzelnen Patienten effizientere Therapie einzuführen. Wichtig ist dabei festzustellen, dass die verwendeten physiologischen und laborchemischen Parameter jeweils indirekte bzw. Surrogatparameter für die Aktivität der MR-Funktion sind. In der Zusammenschau dieser Parameter wird es möglich, den Einfluss des MR auf die zu untersuchenden funktionellen Systeme genauer zu definieren. Im Folgenden wird bei jedem Parameter genauer erläutert in welchem Zusammenhang dieser steht und welche Aussagekraft er dadurch haben kann.

Die zu bestimmenden physiologischen Parameter und deren Zusammenhang mit dem RAAS sind folgende:

- Messung von **Kortisol und Aldosteron** zum Zeitpunkt des Aufwachens mittels Entnahme einer **Speichelprobe** mit der Salivette® Cortisol (Sarstedt). Damit besteht die Möglichkeit exakte Analyseergebnisse auch bei kleinen Speichelmengen und/oder sehr niedrigen Kortisol- oder Aldosteronspiegeln zu erhalten.

- Die Konzentration von Aldosteron, dem physiologischen Liganden am MR, ist bei Patienten mit depressiven Störungen erhöht (Murck et al., 2003; Emanuele et al., 2005). Es gibt einige Hinweise, dass die erhöhte Aldosteron-Konzentration nicht nur ein Epiphänomen darstellt, sondern kausal in die Genese depressiver Störungen involviert sein könnte. So finden sich bei Patienten mit einem Hyperaldosteronismus (Conn-Syndrom) häufiger depressive Störungen (Sonino et al., 2011). Ein weiterer klinischer Hinweis bei Patienten mit depressiven Störungen, der in unserem Zusammenhang besonders relevant ist, sind Polymorphismen des Angiotensin-Converting-Enzym-Gens und des Angiotensin-Rezeptor-Gens, welche einen prädiktiven Effekt auf das Ansprechen einer konventionellen antidepressiven Therapie haben. Das Vorhandensein einer aktiveren Variante, die zu einer erhöhten Aldosteronkonzentration führen sollte, ist mit einem schlechteren Ansprechen verbunden (Bondy et al., 2005). Eine Bestätigung für diesen Befund kommt aus dem Tiermodell: die subchronische Gabe von Aldosteron löst depressionsähnliches Verhalten aus. Gleichzeitig konnte festgestellt werden, dass diese subchronische Aldosterongabe im Hippocampus eine Transkriptionsänderung bei Genen hervorruft, die mit Inflammation, glutamaterger Aktivität, synaptischen und Neuronen-remodeling assoziiert sind. Des Weiteren überlappen Aldosteron-regulierende Gene mit solchen Genen, die bei einer Stressreaktion wie dem Forced Swimming Test reguliert werden (Hlavacova et al., 2011).
- Kortisol und Aldosteron haben teilweise überlappende Regulationssysteme: beide Steroide werden durch ACTH freigesetzt (siehe Murck et al., 2012). Für den Zusammenhang zwischen Kortisol und depressiven Störungen gibt es umfangreiche Literatur, z.B. Holsboer, 1999. In unserem Zusammenhang ist von besonderem Interesse, dass der Quotient der Aldosteron/Kortisol-Konzentration einen Hinweis auf das Verhältnis des RAAS vs. des ACTH-Hormons bei der Aldosteronregulation spielt. Ein niedriger Aldosteron/Kortisol-Quotient weist auf einen ausgeprägteren Anteil von ACTH hin, somit auf eine Erhöhung beider adrenaler Hormone. Ein höherer Aldosteron/Kortisol-Quotient dagegen deutet auf einen relativ ausgeprägteren Anstieg von Aldosteron und somit einen vermehrten Einfluss des Renin-Angiotensin-Systems hin.

- Die **Totale-Schlaf-Dauer (TST)** und die **Slow-Wave-Sleep-Dauer (SWS)** werden mit Hilfe eines ambulanten Schlaf-EEGs (Zeo Sleep Manager – Bedside, der Zeo Inc., 320 Nevada Street, Newton, MA 02460) gemessen. Hauptelement ist ein Stirnband mit Elektroden aus leitenden Fasern, die keine Vorbereitung der Haut benötigen und in

kabellosem Kontakt zu einem separaten Monitor neben dem Bett steht. Validierungsstudien mit einer konventionellen Polysomnographie kommen zu dem Ergebnis, dass die TST & SWS des Zeo Gerätes, welches kommerziell im Handel erwerblich ist, mit einem Standard-EEG gut korrelieren (Shambroom et al., 2011).

- Bei depressiven Patienten sind Schlafstörungen und verminderter SWS mit einem hohen Kortisolspiegel verbunden. Ein übergeordneter Regulationsmechanismus scheint dafür verantwortlich zu sein. Insbesondere gibt es Hinweise, dass sowohl ein verminderter Tiefschlaf als auch eine vermehrte Kortisolkonzentration auf eine erhöhte Aktivität am MR zurückzuführen sein könnten (Born et al., 1991, 1997). Des Weiteren besteht ein enger zeitlicher Zusammenhang der Aktivität des RAAS mit Schlafprozessen: die Konzentration von Renin und Aldosteron steigt in Synchronizität mit einem Anstieg des Tiefschlafs (Charloux et al., 1999). Somit bietet die Dauer des SWS ein Korrelat für die Aktivität des MR.

- Die **Herzratenvariabilität (HRV)** und die **Herzfrequenz** werden bei vorgegebener Atemfrequenz mittels eines im kommerziellen Handel erhältlichen Systems kurz nach dem Erwachen für eine Minute mehrfach gemessen und gemittelt. Dabei wird beim Patienten die Pulsrate mit Sensoren abgeleitet. Der Sensor steht über einen speziellen Funkempfänger mit einem iPhone, einem iPod touch oder einem Androidgerät in Verbindung. Eine kommerziell verfügbare Applikation (App) namens iThlete (HRV Fit Ltd, Hants, UK) analysiert die eingegebenen Daten. Das Messvorgehen ist folgendes: Auf Anweisung der App wird der Atem synchronisiert und die Herzfrequenz und HRV über einen Zeitraum von einer Minute gemessen. Der Vorgang wird wiederholt, um die Variabilität zu reduzieren.
  - Depressionen sind mit einem erhöhten Risiko für kardiovaskuläre Ereignisse assoziiert (Carney et al., 1987; Gonzalez et al., 1996). Selbst bei Depressionen ohne eine kardiovaskuläre Begleiterkrankung besteht eine Korrelation mit einer reduzierten HRV, wobei die HRV mit steigender Depressionsschwere abnimmt (Kemp et al., 2010). Die HRV wird durch das RAAS beeinflusst (Schmidt et al., 1999; Ovaert et al., 2010): Akut erhöht Aldosteron die HRV, andererseits steigert die chronische Gabe von MR-Antagonisten die HRV. Sowohl das autonome Nervensystem als auch die Aldosteronsekretion unterliegen Tagesschwankungen, welche sich in morgendlichen Peaks sowohl des Aldosterons als auch der sympathovagalen Balance zeigen. Durch diese Befunde liegt es nahe, dass diese beiden Systeme miteinander in Verbindung stehen (Yee et al., 2001). Es zeigt sich aber auch, dass eine Aldosteronblockade mit einem Aldosteronantagonisten wie Spironolacton eine Erhöhung der morgendlichen Herzfrequenz nach sich

zieht (MacFadyen et al., 1997). Daraus schließen wir, dass die HRV als funktioneller Parameter der MR-Aktivität interpretiert werden kann.

- Der **Blutdruck (RR)** wird mittels einer elektronischen Blutdruckmanschette oder analog gemessen.
    - Mineralokortikoide wie Aldosteron erhöhen den Blutdruck durch Vasokonstriktion, Natrium- und Wasseraufnahme und erhöhte Natriumrückresorption in der Niere. Somit ist weitgehend bekannt, dass der MR an der Regulation des Blutdruckes beteiligt ist. Wesentlich ist jedoch, dass der MR nicht nur in der Peripherie, sondern auch in spezifischen Arealen des ZNS vorkommt, die an der Blutdruckregulation beteiligt sind. Dabei ist insbesondere der Nucleus tractus solitarius zu erwähnen (Geerling and Loewy, 2009).

- Das **Gewicht** und die **Größe** der Patienten werden gemessen.

- Eine **Geschmacksprobe** mit einer 0,9%igen (oder 1M) **Kochsalzlösung** und eine Einschätzung mittels visueller Analogskala zur Salzpräferenz (Eigenentwicklung) werden durchgeführt. Dabei werden zwei Parameter erhoben: 1) Einschätzung der Salzigkeit (0: nicht salzig; 10: extrem salzig); 2) Einschätzung der Aversion (0: sehr angenehmer Geschmack; 10: extrem unangenehmer Geschmack).
    - Wie der Blutdruck wird auch die Salzaufnahme durch Aldosteron und andere MR-Agonisten erhöht (Wolf, 1965; Wolf and Handal, 1966; Fluharty and Epstein, 1983). Auf der anderen Seite reduzieren MR-Antagonisten den Appetit auf Salziges (Sakai et al., 1986; Francis et al. 2001; Sullivan et al., 2004). Patienten, die an einer Depression erkrankt sind, leiden häufig an Appetitlosigkeit, welche durch eine subjektive Geschmackslosigkeit des Essens hervorgerufen sein könnte. Das RAAS ist unter anderem dafür verantwortlich eine Homöostase der Elektrolyte aufrecht zu erhalten. Angiotensin II sorgt zentral bei einer Hyponatriämie dafür, dass Salz aufgenommen wird, indem es den Salzappetit anregt. So zeigt sich in einer heterogenen Gruppe von depressiven Patienten, dass eine erhöhte Kortisolkonzentration im Urin mit einem gesteigerten Appetit zusammenhängt (Casper et al., 1988). Hierbei ist es bei unserer Untersuchung von Wichtigkeit, dass der Salzappetit im ZNS unter dem

Einfluss von Mineralokortikoidrezeptoren steht und damit ein einfacher funktioneller Marker für dessen Funktion sein könnte.

Folgende Laborparameter werden bestimmt:

- Es sollen die Blutelektrolyte, insbesondere die Konzentration von Plasmamagnesium bestimmt werden. Für die Messung von Plasmamagnesium wird auf klinische Blutentnahmen zurückgegriffen.
    - Eine der Hauptaufgaben des Aldosterons ist die Elektrolytregulation. Auch **Magnesium** unterliegt einer Regulation durch Aldosteron. Im Tiermodell zeigt sich, dass akute (Charlton and Armstrong, 1989) und chronische (Horton and Biglieri, 1962) Aldosterongabe zu einer erhöhten Magnesiumausscheidung im Urin führt. Des Weiteren führt beim Menschen die Gabe des MR-Antagonisten Spironolacton zu einer erhöhten Serummagnesiumkonzentration (Barr et al., 1995). Die Erhöhung der Plasmamagnesiumkonzentration bei depressiven Patienten deutet auf eine Störung in der Magnesiumregulation hin. Diese Störung könnte auf der Ebene einer gestörten MR-Funktion vorliegen. Da der MR der Hauptrezeptor für aldosteronregulierende Effekte in der Niere ist, kann eine reduzierte Magnesiumausscheidung trotz einer erhöhten Aldosteronkonzentration vorliegen. Hierdurch zeigt sich eine mögliche Verbindung zwischen dem Magnesiumgehalt im Blut und der Aktivität am MR.
    - Bei depressiven Patienten zeigen sich höhere Plasma- und erythrozytäre Magnesiumspiegel als bei Gesunden (Widmer et al., 1993). Des Weiteren scheint eine Normalisierung des Magnesiumspiegels mit einer Besserung der klinisch symptomatischen Depression einherzugehen (Widmer et al., 1992). Andererseits gibt es epidemiologische Hinweise, dass eine mangelnde Magnesiumzufuhr zu einem Risiko für die Entwicklung von depressiven Störungen führt (Jacka et al., 2009). Auch im Tiermodell wurde gezeigt, dass Magnesiumentzug depressives und ängstliches Verhalten bei Ratten hervorruft, welches sensitiv gegenüber einer Behandlung mit Antidepressiva ist (Singewald et al., 2004). Es konnte in klinischen Einzelbeobachtungen gezeigt werden, dass eine orale Gabe von Magnesium einen antidepressiven Effekt hat, der ähnlich dem starker Antidepressiva war (Eby III and Eby, 20109).
    - Es besteht ein Zusammenhang mit anderen erhobenen Parametern: eine Stress-induzierte Reaktion der HRV korreliert mit dem Magnesiummetabolismus (Takase et al., 2004).

      Jedoch ist die Datenlage zum Zusammenhang zwischen Plasmamagnesium und Depression heterogen. Es scheint so, als seien sowohl ein Anstieg als auch ein

Abfall des Serummagnesiums mit depressiven Syndromen verbunden. An diesem Punkt darf man nicht vergessen, dass depressive Störungen eine heterogene Gruppe von Erkrankungen darstellen. Ein Anliegen dieser Studie ist es diese Heterogenität besser zu charakterisieren.

Zusammengefasst ist Aldosteron über die Aktivierung des MR an der Magnesiumausscheidung beteiligt. Aus diesem Grund kann die Plasmamagnesiumkonzentration als Surrogatparameter für die MR-Aktivität angesehen werden.

- **CRP** soll als inflammatorischer Marker bestimmt werden. Dazu wird auf die klinische Routinebestimmung zurückgegriffen.
    - Bei depressiven Patienten finden sich vermehrt entzündliche Veränderungen. Vor allem zeigt sich diese Veränderung bei CRP und IL-6, etwas weniger findet diese Veränderung auch bei IL-1 statt (Pace et al., 2006; Howren et al., 2009). Wie oben bereits erwähnt stehen Depressionen in engem Zusammenhang mit der koronaren Herzkrankheit, bei der eine entzündliche Komponente angenommen wird (Carney et al., 1987; Gonzalez et al., 1996). Das RAAS weist eine Verbindung zu inflammatorischen Veränderungen auf. So beeinflusst das RAAS Entzündungsparameter im Plasma (Duprez, 2006). Durch eine MR-Blockade mittels Spironolacton konnte im Tiermodell gezeigt werden, dass bei Ratten ein reduzierter Plasmaspiegel von TNF-α nach einem Koronararterienverschluss vorliegt (Francis et al., 2003; Kang et al., 2006). Der Mechanismus über den der MR mit dem inflammatorischen System in Verbindung steht, ist noch nicht völlig klar. Jedoch lassen diese Ergebnisse darauf schließen, dass die Konzentration des Plasma-CRP mit der MR-Funktion in Verbindung steht. Dieser Zusammenhang ist in unserem Kontext insbesondere deshalb relevant, da eine Erhöhung proinflammatorischer Zytokine mit einer Therapierefraktärität bei einer Behandlung mit Escitalopram und möglicherweise auch mit anderen SSRIs in Verbindung steht (Eller et al., 2008).

Bei der Analyse der Blutelektrolyte und der inflammatorischen Marker wird versucht die Blutentnahmen an die routinemäßigen Abnahmen auf Station zu koppeln. Für die Analyse der Blutelektrolyte sowie der inflammatorischen Marker werden 20 ml venöses Blut benötigt.

- Die Entnahme von Plasma zur Bestimmung von **Renin** wird unmittelbar nach der Abnahme von Speichel sowie der HRV-Analyse durchgeführt. Diese zeitliche Abfolge ist vorgesehen, um durch den möglichen Stress durch die Blutentnahme die genannten Parameter nicht zu beeinflussen.
  - Renin ist ein wesentliches Element der Aldosteronfreisetzung (neben ACTH). Die Reninfreisetzung wird über eine negative Rückkopplung mit Angiotensin II am juxtaglomerulären Apparat der Niere reguliert. Insofern kann die Reninkonzentration und insbesondere das Verhältnis von Aldosteron zu Renin einen Hinweis auf die Aktivität des RAAS darstellen.

- **Genexpressionsmuster von mononukleären Zellen** des peripheren Blutes. Vollblut wird in spezielle Teströhrchen abgenommen und der Genexpressionsanalyse zugeführt. Diese Analyse wird von Covance (Princeton, USA) unterstützt und ausgeführt. Dabei soll keine Asservierung der DNA-Proben im Rahmen einer Biomaterialbank stattfinden. Die Blutproben werden nach der Analyse direkt bei Covance vernichtet.
  - Wie oben beschrieben gibt es neben der Korrelation zwischen erhöhten inflammatorischen Veränderungen und Depressionen auch eine Korrelation zu einer Therapierefrakterität bei einer Behandlung mit SSRIs (O'Brien et al., 2007; Eller et al., 2008). Hier soll die Frage beantwortet werden, inwieweit Veränderungen der Genexpression, wie sie im Tiermodell unter Stress und unter Aldosterongabe gefunden wurden, sich auch im Menschen widerspiegeln. Interessant ist, dass Stress und Aldosterongabe im Tiermodell zu ähnlichen Veränderungen der Genexpression im Hippokampus führen, einem dicht mit MR besetzten Teil des Gehirns, der eng in die Stressregulation mit einbezogen ist. Dabei hat ein großer Teil der beobachteten Genexpressionsveränderungen Bezug zum inflammatorischen System (Hlavacova et al., 2011). Somit könnte die Untersuchung peripherer mononukleärer Zellen und anderer Marker, wie das CRP, die an einer Inflammation beteiligt sind, direkt den Einfluss der MR-Aktivität abbilden. Dies gild sofern Überlappungen zwischen Veränderungen im ZNS und in Leukozyten angenommen werden (Ahokas et al., 2003; Gerling et al., 2003). Für die Analyse der Genexpression ist eine Entnahme von 5 ml venösen Blutes pro Zeitpunkt nötig.

Alle beschriebenen Parameter stehen mit der Aktivität des MR und des RAAS in Verbindung. Der am direktesten zugängliche Parameter ist die Konzentration von Speichelaldosteron. Es besteht die Hypothese, dass eine hohe Aldosteronkonzentration mit einer Non-Response auf konventionelle Antidepressiva einhergeht. Alle weiteren Parameter sind, wie beschrieben, indirekt und als Surrogatparameter der MR-Aktivierung zu betrachten. Aus dem oben

Beschriebenen lässt sich ableiten, dass eine erhöhte Aktivität des MR mit spezifischen Veränderungen einhergeht und aus diesem Grund gleichfalls Prädiktoren von Therapie-Non-Response sein sollten.

Diese Parameter sind:

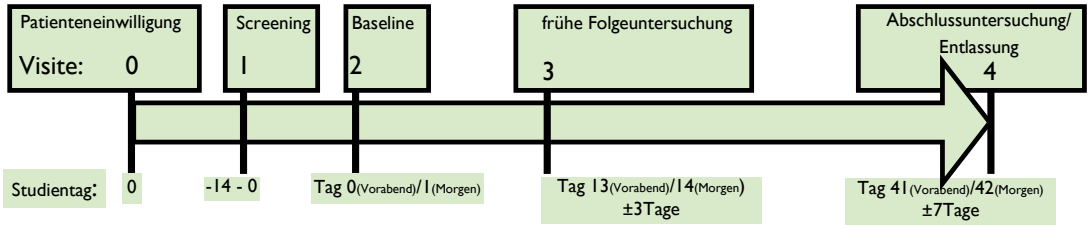
1. Hohe Tiefschlaf- und Gesamtschlafdauer
2. Relativ niedrige Kortisolkonzentration, z.B. im Rahmen eines hohen Aldosteron/Kortisol-Quotienten
3. Niedrige Herzratenvariabilität (HRV)
4. Hoher arterieller Blutdruck
5. Hohes Körpergewicht (relativ zur Körpergröße durch Wassereinlagerung (body mass index, BMI))
6. Niedrige Plasmamagnesiumkonzentration
7. Hohe CRP-Konzentration
8. Plasmareninkonzentration: niedrige Reninkonzentration als Marker für ausgeprägte Rückkopplung durch Angiotensin und Aldosteron
9. Hoher Salzappetit, ausgedrückt durch verminderte Sensitivität für salzigen Geschmack und weniger aversives Rating

Neben dem konfirmativen Aspekt, für den die Aldosteronkonzentration als Zielparameter angesehen wird, wird in explorativer und Hypothesen-generierender Weise geprüft, welcher der genannten Parameter, entweder einzeln oder in Kombination, zu einer Verbesserung der Sensitivität und Spezifität der Vorhersage führen kann.

Außerdem wird getestet, inwieweit diese biologischen Parameter mit klinischen Charakteristika im Zusammenhang stehen. So lässt etwa eine Zusammenschau der genannten negativen Prädiktoren für Therapie-Response vermuten, dass ein starker Zusammenhang mit der sogenannten atypischen Depression besteht (Murck et al., 2012).

Studienablauf

Alle im Folgenden beschriebenen Visiten sind in der Tabelle 1 noch einmal übersichtsweise aufgelistet und auf folgendem Zeitstrahl visualisiert:



1) Screening (Visite 0)

Alle neu aufgenommenen Patienten der Klinik für Psychiatrie und Psychotherapie der Philipps-Universität Marburg, die den Einschlusskriterien dieser Studie entsprechen bzw. den Ausschlusskriterien nicht widersprechen, werden nach ausführlicher ärztlicher Aufklärung gefragt, ob sie an der Studie teilnehmen möchten. Die Patienten erhalten während dieses Gespräches die Patienteninformation sowie die Einwilligungserklärung. Falls Fragen aufkommen, werden diese ausführlich beantwortet. Die Patienten haben soviel Zeit wie sie wünschen einer Teilnahme zuzustimmen bzw. zu widersprechen.
Erst wenn ein Patient eingewilligt hat, folgt die Visite 1.

2) Screening (Visite 1)

Hat ein Patient eingewilligt, wird bei der ersten Visite als erstes überprüft, ob die Diagnosen noch mit den Ein- bzw. Ausschlusskriterien vereinbar sind. In einem Gespräch werden die internistischen Diagnosen sowie die Medikation anamnestisch erhoben. Des Weiteren werden die soziodemographischen Daten (inkl. Alter, Geschlecht, Größe) mit einem Fragebogen erfasst und eine Nikotin-, Alkohol- sowie Familienanamnese erhoben.
Sind zu diesem Zeitpunkt immer noch alle Ein- bzw. Ausschlusskriterien erfüllt, findet zum nächstmöglichen Zeitpunkt die Visite 2 statt.

3) Baseline (Visite 2)

Zwischen der Visite 1 und der Visite 2 sollten nicht mehr als 14 Tage liegen. Zu Beginn des eigentlichen Studienabschnittes, dem Vorabend des Tages der Baseline-Erhebung (Tag 1), wird der Patient visitiert. Es wird überprüft, ob die Ein- und Ausschlusskriterien noch für ihn gültig sind und es werden folgende Fremdbeurteilungsfragebögen mit dem Patienten erhoben: HDRS-21, BPRS, CGI und GAF. Des Weiteren wird eine Änderung der Medikation dokumentiert. Danach wird dem Patienten erklärt, wie die Schlaf-EEG-Untersuchung

durchgeführt wird. Im Verlauf der Nacht wird das Schlaf-EEG abgeleitet, wobei der Patient in seiner gewöhnlichen Umgebung auf Station verbleibt. Dazu legt sich der Patient das Stirnband mit den EEG-Elektroden selbständig an, ggf. mit Hilfe des Pflegepersonals. Die Ableitung wird automatisch aktiviert.

Am Morgen des Folgetages wird der Patient um 7 Uhr visitiert. Als erstes wird Speichelaldosteron und -kortisol mithilfe der Salivette® Corstisol von Sarstedt gesammelt. Im Anschluss findet die Analyse der HRV statt. Dazu hält der Patient die Ableitelektroden in beiden Händen. Das Pulssignal wird kabellos an ein entsprechend ausgerüstetes iPhone (oder Äquivalent) weitergeleitet. Eine Ableitung dauert eine Minute, während dieser der Patient einen vorgegebenen Atemrhythmus einhalten soll. Die Anweisung zur Atmung wird vom Display des iPhones abgelesen. Die Messung wird dreifach mit einem Intervall von ca. 1 Minute wiederholt. Es werden der Puls und der Blutdruck gemessen. Danach wird die Geschmacksprobe durchgeführt. Eine definierte Salzlösung (0,9%) wird dem Patienten in den Mund gegeben. Mittels zweier visueller Analogskalen (Salzigkeit, angenehm vs. unangenehm) wird die Wahrnehmung des Patienten erhoben. Nach diesen Erhebungen wird dem Patienten zur Bestimmung von Plasmarenin Blut abgenommen. Zum Schluss werden dem Patienten die Selbstbeurteilungsfragebögen QIDS-SR-16 und BDI vorgelegt und der Patient füllt die Skalen gemäß den Erläuterungen aus. Des Weiteren wird das Gewicht vor dem Frühstück gemessen. Es wird das Schlaf-EEG ausgelesen.

4) Frühe Folgeuntersuchung (Visite 3)

Am Tag 13 nach Baseline, dem Vorabend des Tages 14 nach der Visite 2 plusminus 3 Tage, wird der Patient besucht. Es werden folgende Fremdbeurteilungsfragebögen mit dem Patienten durchgeführt: HDRS-21, BPRS, CGI und GAF. Des Weiteren wird nach einer Änderung in der Medikation gefragt bzw. im Stationsprotokoll nachgeschlagen. Danach wird dem Patienten noch einmal erklärt, wie das Schlaf-EEG korrekt angewendet wird.

Am Morgen des Folgetages wird der Patient um 7 Uhr visitiert. Als erstes wird ihm Speichelaldosteron und -kortisol mithilfe der Salivette® Cortisol von Sarstedt abgenommen. Im Anschluss findet die Analyse der HRV statt. Es werden der Puls und der Blutdruck gemessen. Danach wird die Geschmacksprobe durchgeführt. Nach diesen Erhebungen wird dem Patienten Blut abgenommen. Zum Schluss wird der QIDS-SR-16 Fragebogen vom Patienten ausgefüllt.

Das Gewicht wird vor dem Frühstück gemessen. Es wird das Schlaf-EEG ausgelesen.

5) Abschlussuntersuchung (Visite 4)

Die Visite 4 stellt die letzte Visite dar. In der Regel wird der Patient am Tag 41 nach Baseline, dem Vorabend des Tages 42 nach der Visite 2 (Baseline) plusminus 7 Tage, besucht. Im Falle einer wesentlichen Umstellung der Medikation/Behandlung oder im Falle einer frühzeitigen Entlassung des Patienten wird diese Untersuchung vorgezogen bzw. zusätzlich durchgeführt.

Bei der Abschlussuntersuchung findet genau das gleiche Untersuchungsprogramm statt, wie zum Baseline-Zeitpunkt (Visite 2):

Es werden folgende Fremdbeurteilungsfragebögen mit dem Patienten durchgeführt: HDRS-21, BPRS, CGI und GAF. Des Weiteren wird nach einer Änderung in der Medikation gefragt bzw. im Stationsprotokoll nachgeschlagen. Danach wird dem Patienten noch einmal erklärt, wie die Schlaf-EEG-Untersuchung durchgeführt wird. Im Verlauf der Nacht wird das Schlaf-EEG abgeleitet, wobei der Patient in seiner gewöhnlichen Umgebung auf Station verbleibt. Dazu legt sich der Patient, eventuell mit Hilfe des Pflegepersonals, das Stirnband mit den EEG-Elektroden selbständig an. Die Ableitung wird automatisch aktiviert.

Am Morgen des Folgetages wird der Patient um 7 Uhr visitiert. Als erstes wird Speichelaldosteron und -kortisol mithilfe der Salivette® Cortisol von Sarstedt gesammelt. Im Anschluss findet die Analyse der HRV statt. Dazu hält der Patient die Ableitelektroden in beiden Händen. Das Pulssignal wird kabellos an ein entsprechend ausgerüstetes iPhone (oder Äquivalent) weitergeleitet. Eine Ableitung dauert eine Minute, während dieser der Patient einen vorgegebenen Atemrhythmus einhalten soll. Die Anweisung dazu wird vom Display des iPhones abgelesen. Die Messung wird dreifach mit einem Intervall von ca. 1 Minute wiederholt. Es werden der Puls und der Blutdruck gemessen. Danach wird die Geschmacksprobe durchgeführt. Eine definierte Salzlösung (0,9%) wird dem Patienten in den Mund gegeben. Mittels zweier visueller Analogskalen (Salzigkeit, angenehm vs. unangenehm) wird die Wahrnehmung des Patienten erhoben. Nach diesen Erhebungen wird dem Patienten Blut abgenommen zur Plasmareninbestimmung. Zum Schluss werden dem Patienten die Selbstbeurteilungsfragebögen QIDS-SR-16 und BDI vorgelegt und der Patient füllt die Skalen nach Anweisung aus. Des Weiteren wird das Gewicht vor dem Frühstück gemessen. Es wird das Schlaf-EEG ausgelesen.

Tabelle 1 listet die oben beschriebenen Untersuchungen auf und zeigt zu welchen Zeitpunkten sie durchgeführt werden.

	Screening	Screening	Baseline (vor Umstellung der Medikation an Tag 1)	Folgeuntersuchungen	
Visite	0	1	2	3	4
Studientag		Tag -14- 0	Tag 0/1	Tag 13/14 ± 3 Tage	Tag 41/42 ± 7 Tage/Entlassung
Patienteneinwilligung	X				
Diagnosebestätigung (klinisch)		X	X		
Relevante internistische Diagnosen		X			
Erhebung psychiatrische Medikation		X	X	X	X
Erhebung nicht-psychiatrische Medikation		X	X	X	X
Soziodemographische Daten		X			
Alkohol- und Nikotinanamnese		X			
Geschlecht, Alter, Gewicht, Größe		X	X (Gewicht)	X (Gewicht)	X (Gewicht)
Familienanamnese		X			
Zustandsbarometer (3x täglich)					
Schlaf-EEG-Anlage			X Vorabend (Abend Tag 0)	X (Vorabend)	X (Vorabend)
Routineblutentnahme			X	X	X
Speichelprobe nach dem Aufwachen			X Morgen (Tag 1)	X (Morgen)	X (Morgen)
HRV-Analyse			X Morgen (Tag 1)	X (Morgen)	X (Morgen)
Blutdruck und Herzfrequenz			X Morgen (Tag 1)	X (Morgen)	X (Morgen)
Skalen: HDRS-21, CGI, BPRS, GAF			X (Abend Tag 0)	X (Vorabend)	X (Vorabend)
QIDS-SR-16			X Morgen (Tag 1)	X (Morgen)	X (Morgen)
BDI			X		X
Geschmack-VAS			X Morgen (Tag 1)	X (Morgen)	X (Morgen)
Blutentnahme Genexpression			X (Morgen)	X (Morgen)	X (Morgen)

Neuheit

Gelöste und ungelöste einschlägige Probleme in der Literatur

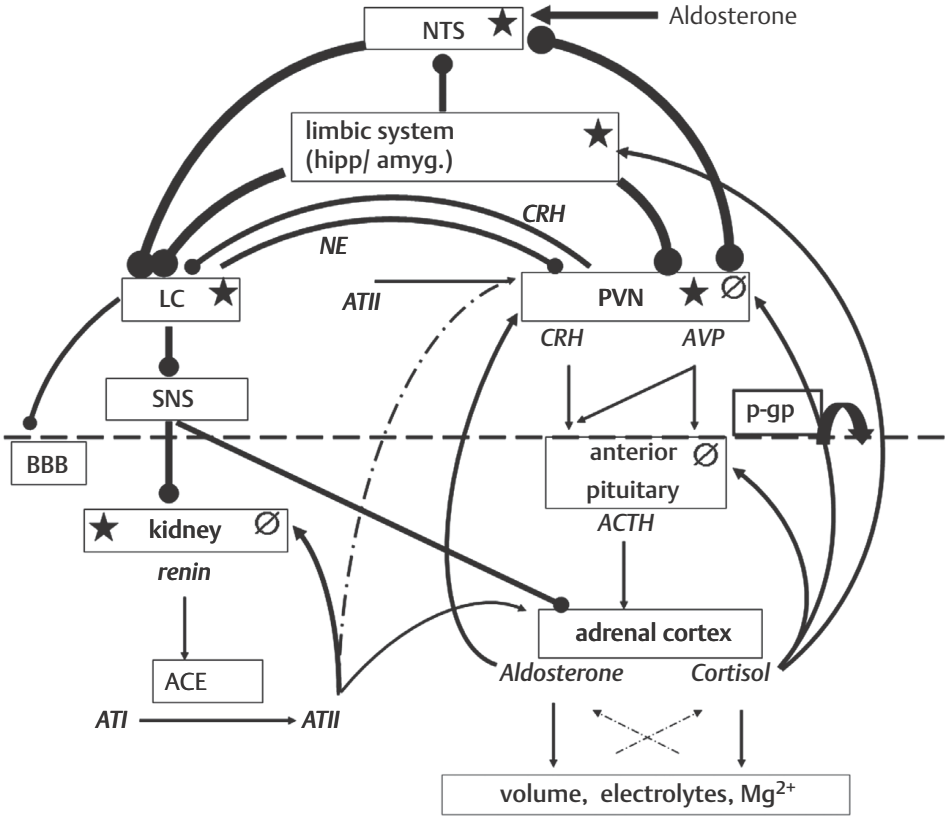
Das vorherrschende Erklärungsmodell für die mögliche Wirksamkeit von antidepressiver Therapie basiert auf einer Dysfunktion monoaminerger Systeme. Dieses Model wurde zur Erklärung von Substanzen, die vor mehr als 50 Jahren auf den Markt eingeführt wurden, etabliert. Parallele Erklärungsmodelle fassen depressive Störungen als stressbedingte Erkrankungen auf und fokussierten sich auf die Modifikation des Stresshormonsystems und dabei insbesondere auf den gut charakterisierten Hyperkortisolismus bei schweren, sogenannten melancholischen depressiven Patienten (Holsboer, 1999). In neuerer Zeit wurde die Bedeutung von inflammatorischen Prozessen (Zorrilla et al., 2001) und Veränderungen der glutamatergen Neurotransmission (Krystal et al., 2002) hervorgehoben. Eine umfassende Theorie, die allgemein anerkannt wäre, besteht bislang nicht.

Ein übergreifendes System mit Verbindung sowohl zu monoaminerger und glutamaterger Transmission als auch mit Verbindungen zu inflammatorischen sowie Stress-regulierenden Systemen ist hingegen das RAAS (zusammengefasst von Murck et al., 2012). Erst vor wenigen Jahren wurde deutlich, dass ein Hyperaldosteronismus bei bestimmten Patienten mit depressiven Störungen besteht (Murck et al., 2003; Emanuele et al., 2005). Eine erhöhte Aktivität des RAAS wurde bei unter Stress stehenden Menschen (Makatsori et al., 2004) und im Tiermodell (Grippo et al., 2005) beschrieben. So führt eine subchronische Aldosteron-Gabe zu depressionsähnlichem Verhalten (Hlavacova et al., 2011). Des Weiteren finden sich Verhaltensauffälligkeiten bei Patienten mit einem durch ein Adenom hervorgerufenen Hyperaldosteronismus, welche sich insbesondere durch eine depressive Stimmung sowie durch Angst äußern (Sonino et al., 2011).

Somit ist plausibel, dass das RAAS für die Pathophysiologie und Behandlung von depressiven Störungen bedeutsam ist. Dieser Ansatz beruht nicht auf Hypothesen, die das klassische monoaminerge Systeme einbeziehen. Insofern besteht die Möglichkeit, dass Patienten, die auf klassische monoaminerge Medikamente ansprechen, sich von Non-Responder unterscheiden. Hierbei könnte die interindividuelle Reaktion des RAAS eine Rolle spielen. Dieser Sachverhalt soll in dieser Studie untersucht werden.

Abbildung 1 zeigt die komplexe Interaktion der untersuchten neuroendokrinen Systeme sowie deren Zusammenhang mit dem vegetativen (sympathischen) Nervensystem. Aufgrund dieser Komplexität sind vielfältige Parameter und deren Beziehung zueinander notwendig, um das System funktionell beschreiben zu können.

Abbildung 1 (Murck et al., 2012): Die Abbildung zeigt eine Übersicht der Hauptsysteme, welche bei der Pathophysiologie der Depression und bei dem Renin-Angiotensin-Aldosteron-System verbunden sind. Die „drumsticks“ repräsentieren neuronale Verbindungen, wobei diese nicht unbedingt monosynaptischen Verbindungen entsprechen; Pfeile stellen endokrine Einflüsse dar und gestrichelte Pfeile zeigen indirekte Mechanismen. PVN: Nucleus paraventricularis; NTS: Nucleus of the solitary tract; CRH: Corticotropin Releasing Hormone; NE: Norepinephrine; ATII: Angiotensin II; ACTH: Adrenocorticotropin; BBB: Blood Brain Barrier; LC; Locus coeruleus; SNS: Sympathetic Nervous System; AVP: Arginine vasopressine; ACE: Angiotensin Converting Enzyme; p-gp: p-glycoprotein.



Welches dieser Probleme wird angegangen?

Konkret wird der neurobiologische Zusammenhang der Symptome mit dem RAAS und der Einfluss der gängigen klinischen Therapieverfahren auf das RAAS untersucht.
Dabei werden die o.g. Parameter gemessen und in Zusammenhang mit den klinisch-psychiatrischen Erfassungen mittels Symptomerfassung in Selbst- und Fremdratings und Lebensqualitätsbögen gesetzt.

Im Einzelnen sollen folgende Hypothesen untersucht werden:

1. Eine funktionelle Überaktivität des Mineralokortikoidrezeptors (MR) verhindert eine Besserung der depressiven Symptomatik, insbesondere bei Behandlung mit SSRIs/SNRIs.
2. Das frühzeitige Auftreten (1-2 Wochen nach Therapiebeginn) von Markern einer verminderten MR-Aktivierung ist ein prädiktiver Marker für eine positive Response.
3. Eine unveränderte MR-Aktivierung deutet auf eine Therapierefraktärität hin.

Gründe dafür?

Die Erfassung der o.g. klinischen-psychiatrischen Verlaufsparameter ist notwendig, um die depressive Symptomatik messbar zu machen. Es handelt sich durchweg um in der psychiatrischen Literatur gebräuchliche Standardverfahren.
Die Erfassung o.g. physiologischer und neurobiologischer Parameter ergibt sich aus folgenden Überlegungen:
Depressive Störungen sind eine heterogene Gruppe von Erkrankungen. Verschiedene biologische Parameter zur Differenzierung sind beschrieben worden. Diese Parameter sollten jedoch nicht getrennt betrachtet werden, da sie in funktionellem Zusammenhang stehen. So sind etwa bei depressiven Patienten Schlafstörungen und verminderter SWS mit einem hohen Kortisolspiegel verbunden. Ein übergeordneter Regulationsmechanismus scheint dafür verantwortlich zu sein. Bei Gesunden ist vermehrter Tiefschlaf mit der Freisetzung von Renin und Aldosteron verbunden. Bei depressiven Patienten erscheint die Regulation dieses Systems gestört zu sein, da Schlafentzug bei depressiven Patienten im Vergleich zu Gesunden nicht zu einem SWS- und Aldosteron- (jedoch Renin-) Anstieg führt. Die HRV verändert sich im Verlauf der Nacht in Abhängigkeit von den Schlafstadien (Charloux et al., 1999; Viola et al., 2002). Außerdem wird die HRV kurzzeitig durch Aldosteron erhöht (was als klinisch wünschenswert erachtet werden kann). Andererseits jedoch auch durch eine langfristige Blockade des MR, was auf gegensätzliche Effekte von akuter und chronischer Wirkung hindeutet. Dieser Befund deutet auch auf eine pathogenetische Komponente von chronisch erhöhtem Aldosteron hin.

Das RAAS weist eine Verbindung zu inflammatorischen Veränderungen auf. Auch bei einer Depression verändern sich unter anderem Entzündungsparameter. Es entsteht eine Leukozytose (mit möglicher relativer Neutrophilie und Lymphopenie). Ein geringfügig erhöhter CD4/CD8-Quotient liegt vor. Es findet sich ein erhöhter Spiegel des CRP, des IL-1 und des IL-6, sowie des PGE_2 welches durch COX-1 und -2 entsteht. Aldosteron kann eine erhöhte Expression der COX-2 bewirken und ist somit proinflammatorisch.

Somit dienen alle genannten Parameter der spezifischen Charakterisierung der neurobiologischen Veränderungen eines Patienten. Als von zentraler Relevanz wird die Aktivität des MR angesehen, der alle genannten Parameter beeinflusst, wie oben ausführlich beschrieben.

Das dargestellte Projekt hat mehrere wesentliche innovative Charakteristika:

- Das Projekt stellt mit einfachen technischen Mitteln biologische Parameter im Verlauf der Behandlung depressiver Patienten dar. Die technische Einfachheit ist ein wesentliches Charakteristikum, das es ermöglicht, eine größere Anzahl von Patienten im Verlauf zu untersuchen, um damit biologische und klinische Subgruppen differenzieren zu können. So soll ein Weg eröffnet werden dem Kliniker einfache klinisch relevante Parameter an die Hand zu geben, um so den Weg zur Benutzung von Biomarkern im klinischen Alltag zu ermöglichen.
- Das Projekt involviert eine Anzahl von Verfahren und Parametern, die in der Zusammenschau deutlich aussagekräftiger sein sollten als einzelne Parameter. Dieser Netzwerkaspekt fokussiert im Wesentlichen auf die Interaktion verschiedener Systeme im Gegensatz zur Ansicht eines „final common pathways“. Die technische Einfachheit der Marker ermöglicht diesen Netzwerkansatz.
- Im engeren Sinne fokussiert das Projekt auf funktionelle Systeme, die einen Bezug zur Regulation der HRV, der Schlafregulation, des RAAS, der klassischen Hypothalamus-Hypophysen-Nebennierenrinden-Achse und dem inflammatorischen System haben. Damit schließt es an frühere Befunde an. Der theoretische Rahmen ist kürzlich ausführlich dargestellt worden (Murck et al., 2012).

Versuchsplanung – biometrisches Studiendesign

Folgende spezifische Hypothesen sollen untersucht werden:
Hypothese 1: Eine funktionelle Überaktivität des Mineralokortikoidrezeptors (MR) verhindert eine Besserung der depressiven Symptomatik, insbesondere bei Behandlung mit SSRIs/SNRIs.
Hypothese 2: Das frühzeitige Auftreten (1-2 Wochen nach Therapiebeginn) von Markern einer verminderten MR-Aktivierung ist ein prädiktiver Marker für eine positive Response.
Hypothese 3: Eine unveränderte MR-Aktivierung deutet auf eine Therapierefraktärität hin.

Patientenzahl

Durch die Einfachheit der zu erhebenden Parameter soll der Einschluss einer möglichst großen Teilnehmerzahl ermöglicht werden. Es wird mit einem Einschluss von ca. 100 Patienten gerechnet (siehe unten, Fallzahlberechnung).

Studienkollektiv

Die **Einschlusskriterien** umfassen folgende Diagnosen der ICD-10:

- F 32 (Depressive Episode)
- F 33 (Rezidivierende depressive Störung)
- F 34 (Anhaltende affektive Störung).

Eine dieser Diagnosen muss der primäre Grund für den aktuellen stationären Aufenthalt in der Klinik für Psychiatrie und Psychotherapie des Universitätsklinikums Marburg sein. Die Begründung des stationären Aufenthaltes durch eine andere psychiatrische Haupterkrankung mit einer der o.g. komorbiden Erkrankungen führt nicht zum Einschluss in diese Verlaufsbeobachtung.

Ausschlusskriterien:

- ICD-10 F 20 bis F 23 (Schizophrene und wahnhafte Störungen)
- Fehlende deutsche Sprachkenntnisse
- Neurologische Erkrankungen, bei denen eine Beteiligung des zentralen Nervensystems bekannt ist, wie z.B. Epilepsie, Speicherkrankheiten oder schwere geistige Behinderung, stellen generell ein Ausschlusskriterium dar.
- Nicht-Einwilligung oder Unfähigkeit zur Einwilligung in die Studie

Statistisches Auswertungsverfahren

Bei dem Studiendesign handelt es sich um eine Kohortenstudie. Dabei wird nur der Verlauf bzw. die Prognose beobachtet. Es findet keine Intervention statt. Es werden drei Gruppen von Parametern bestimmt.

Wir haben im Zusammenhang mit dem Vorhaben eine kostenlose statistische Beratung durch das Institut für Medizinische Biometrie und Epidemiologie in Anspruch genommen. Dabei hat uns Dr. rer. nat. Sebastian Irle beraten und sich an der Formulierung des Abschnittes beteiligt.

Die Parameter und die Messzeitpunkte

1. **Klinische Parameter**, welche die Schwere und den Behandlungsverlauf determinieren können und eine biologische Grundlage haben, sind: Schweregrad, Dauer der Erkrankung und Dauer der depressiven Episode, Alter, Geschlecht, spezifische klinische Merkmale (Vorhandensein von vegetativen Parametern 1. der melancholischen Depression, 2. der atypischen Depression oder 3. die Abwesenheit von vegetativen Parametern). Dabei handelt es sich um nominal gemessene Werte. Die klinischen Parameter dienen zur Einteilung in drei Kohortengruppen.

2. **Biologische Parameter**, die im Verlauf des Projektes als Einflussgrößen metrisch erfasst werden, sind:

a) Speichelaldosteron (bei Erwachen)
b) Tiefschlafdauer (SWS)
c) Morgendliche Herzratenvariabilität (HRV)
d) Plasmamagnesium
e) Arterieller Blutdruck und Pulsfrequenz
f) Speichelkortisol (bei Erwachen)
g) Plasmarenin
h) Inflammatorische Marker, insbes. CRP
i) Salzpräferenz (VAS)
j) Genexpressionsmuster der weißen Blutzellen

3. **Verlaufsparameter**, die im Verlauf des Projektes metrisch erfasst werden und das Outcome bestimmen, sind:

a) Veränderung des Hamilton Depression Rating Scale-6 (HDRS-6) Subscores (Bech, 2006). (Anmerkung: der HDRS-6 Subscore enthält die zentralen depressiven

Symptome, jedoch nicht die vegetativen Symptome. Die Differenzierung in die vegetativen Symptomgruppen macht es notwendig, die vegetativen Symptome aus der Verlaufsanalyse der Besserung der depressiven Symptomatik auszuschließen)

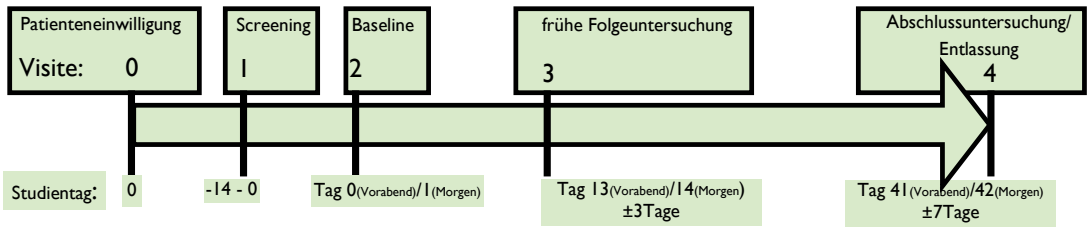
b) Frühe Response (mindestens 20 % Reduktion des HDRS-6 Subscores (der HDRS-21) nach 1-2 Wochen).

c) Response nach 6 Wochen (mindestens 50 % Reduktion des HDRS-6 Subscores des HDRS-21)

d) In explorativer Weise und als Sensitivitätsanalyse der Hauptzielgrößen (Response und Non-Response) wird der Verlauf mit weiteren Skalen in gleicher Weise bestimmt. Diese sind HDRS-21, HDRS-Item 1, QIDS-SR-16, BDI, GAF, CGI und die Auswertung des Zustandsbarometers.

4. **Messzeitpunkte**

Wie auf dem Zeitstrahl visualisiert, werden die Parameter der Punkte 1. - 3. zu drei Zeitpunkten (Visite 2, 3 und 4) der Studie erhoben. Eine exakte Beschreibung der Messzeitpunkte findet sich weiter oben in diesem Protokoll.



Geplante statistische Analysen:

Hypothese 1

Mit Hilfe eines multivariaten Regressionsmodells soll der Einfluss der Parameter der MR-Aktivität zum Baseline Zeitpunkt auf den klinischen Ausgang getestet werden. Dieser wird im Regelfall als die Veränderung des HDRS-6-Wertes zwischen Baseline und dem Zeitpunkt 6 Wochen nach Baseline definiert. Ausnahmen: Falls innerhalb dieses Beobachtungszeitraumes ein wesentlicher Wechsel des Therapieverfahrens durchgeführt wird, werden die Erhebungen unmittelbar zuvor durchgeführt. Des Weiteren, falls die Entlassung des Patienten vor den 6-Wochen-Zeitraum fällt, werden die Erhebungen unmittelbar vor der Entlassung durchgeführt.

Für die Analyse werden drei Gruppen auf Grundlage der Höhe des Rankings der Aldosteronkonzentration definiert: hohe Aldosteronkonzentration bei Baseline (HBA);

mittlere Aldosteronkonzentration bei Baseline (MBA) und niedrige Aldosteronkonzentration bei Baseline (NBA). Der Einfluss dieser Gruppenvariable auf die Veränderung der HDRS-6 wird als primäre Analyse getestet.

Für diese und alle weiteren Analysen werden Geschlecht und Depressionstyp als covariable Faktoren einbezogen. Als Outcome-Variable dient die Reduktion der HDRS-6.

Im Folgenden werden weitere zu analysierende Einflussfaktoren aufgezählt, die als Surrogatparameter die MR-Aktivität darstellen. Die Gruppen werden dazu aufgeteilt in 1. > Median; 2. ≤ Median. Diese werden nach unten aufgelisteter Priorität hierarchisch ausgewertet (1.=höchste Priorität und 8.= niedrigste Priorität):

1. Tiefschlafdauer (SWS): Hoch vs. Niedrig.
2. Herzratenvariabilität (HRV): Hoch vs. Niedrig.
3. Salzpräferenz: Hoch vs. Niedrig.
4. Plasmamagnesium: Hoch vs. Niedrig.
5. Inflammatorischer Marker CRP: Hoch vs. Niedrig.
6. Speichelkortisol: Hoch vs. Niedrig.
7. Arterieller Blutdruck und Pulsfrequenz: Hoch vs. Niedrig.
8. Plasmarenin: Hoch vs. Niedrig.

Die Parameter 1. bis 8. werden in hierarchisch geordneter Weise ausgewertet. Die Tests gelten als konfirmativ, solange in der Hierarchie zuvor alle Parameter eine signifikante Differenz gezeigt haben. Im anderen Falle gelten die Tests als explorativ.

Der Einfluss von Alter, Dauer der depressiven Episode und Dauer der Erkrankung auf die einzelnen biologischen Parameter wird korrelativ getestet und gegebenenfalls mit in die Analysen einbezogen.

Hypothese 2 und 3:
Bei dieser Analyse wird nur die frühzeitige Veränderung (Baseline zu Visite 1 (nach 1-2 Wochen)) der Aldosteronkonzentration im Speichel als Einflussfaktor auf die Veränderung des HRDS-6 zum Zeitpunkt des Outcomes (in der Regel 6 Wochen nach Baseline) getestet. Es werden drei Gruppen auf Grundlage des Rankings der Aldosteronkonzentration definiert: a) AR (Aldosteronreduktion): Reduktion um ≥ 10% der Aldosteronkonzentration; b) Gruppe ANR (Aldosteron Non-Reduktion), keine Reduktion oder kein Anstieg; c) Gruppe AA (Aldosteronanstieg): Anstieg um ≥ 10% der Aldosteronkonzentration. Dabei wird wie für die Hypothese 1 ein multivariates Regressionsmodell angewendet, in welchem Alter, Geschlecht und Depressionstyp in die Analyse einbezogen werden.

Im Folgenden werden weitere zu analysierende Einflussfaktoren aufgezählt, die als Surrogatparameter die MR-Aktivität darstellen und in der untenstehenden Priorität hierarchisch ausgewertet werden sollen (1.=höchste Priorität und 8.= niedrigste Priorität):

1. Tiefschlafdauer (SWS): Abnahme vs. Zunahme
2. Herzratenvariabilität (HRV): Abnahme vs. Zunahme.
3. Salzpräferenz: Abnahme vs. Zunahme.
4. Plasmamagnesium: Abnahme vs. Zunahme.
5. Inflammatorischer Marker CRP: Abnahme vs. Zunahme.
6. Speichelkortisol: Abnahme vs. Zunahme.
7. Arterieller Blutdruck und Pulsfrequenz: Abnahme vs. Zunahme.
8. Plasmarenin: Abnahme vs. Zunahme

Die Parameter 1. bis 8. werden in hierarchisch geordneter Weise ausgewertet. Die Tests gelten als konfirmativ, solange in der Hierarchie zuvor alle Parameter eine signifikante Differenz gezeigt haben. Im anderen Falle gelten die Tests als explorativ.

Der Einfluss von Alter, Dauer der depressiven Episode und Dauer der Erkrankung auf die einzelnen biologischen Parameter wird korrelativ getestet und gegebenenfalls mit in die Analysen einbezogen.

3. Explorative Analysen:
a) Veränderungen der genannten Parameter der MR-Aktivität zum Zeitpunkt Outcome (6 Wochen nach Baseline, bei Entlassung oder bei wesentlichem Wechsel der Therapie) werden mit denen zum Zeitpunkt der Baseline-Erhebung verglichen. Diese Berechnungen werden in der Gesamtgruppe, in den Aldosterongruppen und zusätzlich nach Geschlecht und nach Depressionssubgruppe getrennt durchgeführt. Dafür wird ein Wilcoxon-Test für verbundene

Stichproben herangezogen. Diese Berechnungen werden in der Gesamtgruppe, in den Aldosterongruppen und zusätzlich nach Geschlecht und nach Depressionssubgruppe getrennt durchgeführt.

b) Die Veränderungen der genannten Parameter werden explorativ mittels Pearson's Korrelationskoeffizient berechnet. Diese Berechnungen werden in der Gesamtgruppe, in den Aldosterongruppen und zusätzlich nach Geschlecht und nach Depressionssubgruppe getrennt durchgeführt.

c) Die Genexpressionsmuster werden explorativ bei Baseline mit denen beim Ausgang nach 6 Wochen verglichen. Diese Berechnungen werden in der Gesamtgruppe, in den Aldosterongruppen und zusätzlich nach Geschlecht und nach Depressionssubgruppe getrennt durchgeführt.
Hierfür wird zunächst ein t-Test für jede mRNA durchgeführt, der untersucht, ob sich die Baseline-Intensitätsrate von der Intensitätsrate nach 6 Wochen unterscheidet. Anschließend werden alle mRNA ausgewählt, die in einem auf dem Chip repräsentierten Pathway enthalten sind und deren p-Wert im t-Test kleiner als 5% gewesen ist. Schließlich wird pro Pathway der Anteil der signifikant veränderten Gene an allen Genen im Pathway bestimmt und mit Hilfe eines exakten Fisher-Tests untersucht, ob sich diese Anteile signifikant von den erwarteten Anteilen unterscheiden.

Fallzahlberechnung:

Die Fallzahlplanung bezieht sich auf die Hauptfragestellung 1. Als Baseline Wert für den Score der HDRS-6 sowie dessen Standardabweichung kann von einem Wert von 12 ± 2 ausgegangen werden (Lecrubier and Bech, 2007; Tourian et al., 2009; Bech et al., 2012). Im Verlauf des Beobachtungszeitraumes wird eine mittlere Verminderung des Scores um 6 ± 2,5 erwartet. Eine klinisch relevante Differenzierung der Gruppen wird angenommen, falls sich die Verminderung des Scores um ≥ 2 Punkte unterscheidet. Die Annahme, dass eine Differenzierung mit einer Differenz von 2 Punkten möglich sei, ist konsistent mit Befunden eines Effektes von Polymorphismen des Angiotensin-Rezeptor-Genes sowie des Angotensin-Converting-Enzym-Genes (Bondy et al., 2005).
Bei einer Fallzahl von 41 pro Gruppe kann mit diesen Annahmen von einer Differenzierung der Gruppen bei einem alpha von 0,05 und einer Power von 80% ausgegangen werden (Fallzahlplanung auf Basis des t-Tests). Insgesamt wird also von einer Fallzahl von 82 Patienten ausgegangen.

Belastung und Risiko

Projektbedingte Handlungen am Patienten, Risiken und Komplikationen

Die Teilnahme an der Studie ist mit nahezu keinem Risiko verbunden. Die Blutentnahme wird nach den Regeln der ärztlichen Praxis nur von ausgebildetem medizinischen Personal durchgeführt. Dabei kann es in seltenen Fällen zu lokalen Hämatomen (Blutergüssen) und/oder Infektionen kommen. In sehr seltenen Fällen kann ein kleiner Hautnerv getroffen werden. Es wird nach Möglichkeit versucht die Blutentnahme zur Bestimmung der Blutelektrolyte und der Entzündungsparameter mit den ohnehin im klinischen Alltag anfallenden Blutentnahmen zu synchronisieren, die normalerweise auf Station im zweiwöchigen Rhythmus stattfinden. So muss für die Studie nach Möglichkeit nicht extra Blut entnommen werden.

Das EEG ist ein praktisch unschädliches Verfahren. Im Rahmen des EEGs werden elektrische Potentiale über ein Stirnband erfasst, wobei keine besondere Vorbereitung der Haut von Nöten ist. Da es sich um eine kabellose Übertragungsform der EEG-Daten an einen Monitor am Bettrand handelt, ist ausgeschlossen, dass die zu untersuchende Person in irgendeiner Form von elektrischen Rückkopplungen durch das Gerät gefährdet ist. Die verwendeten EEG-Geräte sind keine reinen Forschungsgeräte, sondern im kommerziellen Handel erwerbbar und als ungefährlich einzustufen. Die einzige Komplikation, die auftreten kann, sind Druckstellen, die durch langes Liegen auf der Elektrode über Nacht zu Stande kommen könnten. Diese Druckstellen sind jedoch ungefährlich und verschwinden kurz nach Abnahme des Elektrodenstirnbandes. Der vorschriftsmäßige Betrieb wird dadurch gewährleistet, dass Personen, die für die Untersuchung verantwortlich sind, zuvor eine intensive Einarbeitung erhalten haben und auch den Patienten im nächtlichen Umgang mit dem Zeo-Sleep-Manager schulen.

Umgang mit Zufallsbefunden: Als Zufallsbefunde könnten pathologische Laborwerte, pathologische EEG-Parameter oder Herzrhythmusstörungen entdeckt werden. Die Patienten werden in einer Patienteninformation darüber aufgeklärt, dass es sich nicht um eine klinische Diagnostik handelt: „Die hier durchgeführten Untersuchungen sind keine diagnostischen Untersuchungen, das heißt die Daten werden nicht auf das Vorliegen einer Erkrankung analysiert.“ Falls Zufallsbefunde entdeckt werden, wird der Studienleiter informiert und übernimmt die Verantwortung dafür, dass der Proband sofort kontaktiert wird. Um diesen Fall a priori zu berücksichtigen, enthält die Patienteninformation den Passus: „Sollten uns trotzdem Auffälligkeiten wie z.B. Herzrhythmusstörungen in der Herzratenvariabilitätsmessung, steile Wellen in der EEG-Ableitung oder weit über die Norm veränderte Laborwerte auffallen, werden Sie darüber von einem Arzt informiert.“

Abbruchkriterien

Solange die technische Sicherheit der Geräte gewährleistet ist und sichere Verfahrensweisen bei der Durchführung der Untersuchungen eingehalten werden können, kann der Versuch weitergeführt werden. Abbruchkriterium ist der Rückzug der Einwilligung des Patienten.

Datenschutz

Daten anonymisiert?

Die klinischen und neuropsychologischen Daten werden ebenso wie die EEG-Daten durch Angabe eines Buchstabens für die Gruppe und einer fortlaufenden dreistelligen Nummerierung kodiert (pseudonymisiert, z.B. A123). Die ärztliche Schweigepflicht bleibt strengstens gewahrt, die Datenschutzbedingungen werden eingehalten.

Speicherung und Übermittlung?

Die pseudonymisierten Daten werden in der Klinik für Psychiatrie und Psychotherapie des Universitätsklinikums Gießen und Marburg für die Dauer von mindestens 10 Jahren bzw. bis zum Widerruf der Einverständniserklärung ausschließlich auf geschützten Rechnern gespeichert. Mitarbeiter der Klinik für Psychiatrie und Psychotherapie des Universitätsklinikums Marburg werten die Daten aus. Die Datenübermittlung erfolgt dabei ausschließlich über gesicherte Datenleitungen.

Die Schlüsselliste, durch die alle Proben zu den Teilnehmern dieser Studie zurückverfolgt werden können, wird von Frau Sabine Fischer, medizinisch-technische Assistentin der Klinik für Psychiatrie und Psychotherapie der Philipps-Universität Marburg, verwaltet und 5 Jahre nach dem Ende dieser Studie vernichtet. Die Schlüsselliste wird in den Räumlichkeiten des Haus-Betanien (Schützenstraße 49, 35039 Marburg), ein Teil der Klinik für Psychiatrie und Psychotherapie, aufbewahrt.

PD Dr. med. Carsten Konrad

Oberarzt und Projektleiter

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6.6. Patientenaufklärung und Patienteneinwilligung

Informationsblatt

für die Patientinnen und Patienten zur Studie

„Veränderungen des Renin-Angiotensin-Aldosteron-Systems im Verlauf einer stationären Depressionsbehandlung“

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Sehr geehrte Patientin, sehr geehrter Patient,

in diesem Schreiben werden Sie darüber aufgeklärt, um was es in dem Projekt „Veränderungen des Renin-Angiotensin-Aldosteron-Systems im Verlauf einer stationären Depressionsbehandlung“ geht und welche Untersuchungen im Einzelnen durchgeführt werden. Wir bitten Sie um Teilnahme an diesem Projekt.

1. Ziel der Untersuchung

Sie werden um die Teilnahme an dieser Studie gebeten, da Sie unter einem depressiven Syndrom leiden. Ein depressives Syndrom wird durch viele Faktoren beeinflusst, u.a. stressvolle Erfahrungen, eine Veranlagung zu erhöhter Sensibilität auf Stress und weiteren körperlichen Einflussfaktoren, wie hormonelle Veränderungen, Veränderungen des Immunsystems sowie Stoffwechselstörungen. Es sind eine Reihe wirksamer Verfahren bekannt, um Depressionen zu behandeln. Dazu gehören psychotherapeutische Verfahren und medikamentöse Therapien. Dennoch ist das Ansprechen auf eine gegebene Therapie für viele Patienten nicht befriedigend, d.h. es dauert oft zu lange, das richtige Verfahren für einen individuellen Patienten zu finden. Das liegt vermutlich daran, dass das Zusammenspiel von therapeutischen Verfahren mit der Vielzahl der biologischen und persönlichen Faktoren eines einzelnen Patienten nicht gut bekannt ist. Unser Ziel ist es hier, mit einfachen Verfahren wichtige biologische Merkmale zu untersuchen, die den Behandlungserfolg bestimmen können.

Durch Ihre Teilnahme erzielen Sie keinen unmittelbaren Nutzen für sich, es wird auch keine Aufwandsentschädigung gezahlt.

Die Teilnahme an dieser Studie ist freiwillig. Sie können jederzeit und ohne Angabe von Gründen Ihre Einwilligung zurückziehen, ohne dass Ihnen daraus Nachteile entstehen.

2. Beschreibung und Ablauf der Untersuchung

Um Ihren Zustand zu erfassen, werden zu verschiedenen Zeitpunkten verschiedene Aspekte Ihrer Depression in Interviews erhoben und mit Skalen erfasst. Einige der Skalen sind sogenannte Selbsterfassungsskalen, die Sie selber ausfüllen sollen. Die Zeitpunkte der Erfassungen sind vor Beginn Ihrer neuen Behandlung (Baseline), nach ca. 2 Wochen der Behandlung, sowie zum Studienabschluss, in der Regel also nach ca. 6 Wochen der Behandlung, oder alternativ, bei Umstellung der Therapie oder bei Entlassung. Es werden nicht immer alle Fragebögen genutzt. Die Bearbeitung dauert ca. 60-90 min.

Daneben sollen biologische Parameter bestimmt werden, die mit einfachen Mitteln eine bessere Beschreibung Ihres körperlichen Zustandes ermöglichen. Diese erfolgen zum Zeitpunkt vor der neuen Behandlung (Baseline), ca. 2 Wochen danach sowie in der Regel nach ca. 6 Wochen. Im Falle einer wesentlichen Umstellung der Therapie oder Ihrer Entlassung wird diese Untersuchung zum Zeitpunkt dieser Ereignisse erfolgen. In besonderen Fällen können mit Ihrer Zustimmung Zwischenerfassungen erhoben werden, z.B. zu Beginn oder Ende einer besonderen Therapiemaßnahme.

Die folgenden Parameter werden morgens um 7 Uhr bei Ihnen erhoben, nachdem Sie aufgewacht sind.

- Es wird ein Schlaf-EEG abgeleitet, für das Sie lediglich ein Stirnband über Nacht tragen, in dem Elektroden aus leitenden Fasern enthalten sind. Dabei ist in aller Regel keine Vorbereitung der Haut notwendig. Die Elektroden haben einen kabellosen Kontakt zu einem separaten Monitor, der neben dem Bett steht. Die Anlage des Stirnbandes ist nicht schwierig, jedoch kann Ihnen das Pflegepersonal helfen, falls Sie Fragen haben. Innerhalb von 2 Minuten können Sie einen komfortablen Sitz des Stirnbandes erreichen. Am Morgen kann es nach dem Erwachen mühelos abgenommen werden.
- Die Herzratenvariabilität (HRV) wird mittels einer kurzen Messung nach dem Erwachen (je eine Minute) mehrfach gemessen. Dabei werden Sie gebeten, je eine Elektrode in jeder Hand zu halten. Alternativ können Ableitungen vom Brustkorb oder mit einem Fingersensor oder Ohrsensor benutzt werden. Auf die Anweisung, welche auf dem Gerät zu sehen ist, wird der Atem synchronisiert und so die HRV über einen Zeitraum von einer Minute gemessen. Dieser Vorgang wird dreifach wiederholt und die gesamte Messung dauert ca. 5 Minuten.

- Der Blutdruck wird mittels einer elektronischen Blutdruckmanschette oder analog gemessen. Diese Messung dauert ca. 2 Minuten.
- Es werden Speichel und Blut gesammelt. Speichelproben (2 ml) werden morgens unmittelbar nach dem Aufwachen gewonnen, um Stresshormone zu bestimmen. Die Blutentnahmen werden nach Möglichkeit mit den ohnehin im klinischen Alltag anfallenden Blutentnahmen synchronisiert, die bei uns normalerweise im zweiwöchentlichen Rhythmus stattfinden, so dass für die Studie nach Möglichkeit nicht extra gestochen werden muss. Es werden dafür etwa 20 ml Blut entnommen, um z.B. die Konzentration von Blutsalzen oder von Entzündungsmarkern zu bestimmen.
- Außerdem soll das Genexpressionsmuster von Leukozyten bestimmt werden. Wichtig ist hierbei zu verstehen, dass es sich **nicht** um eine Analyse Ihres genetischen Kodes, also Ihres Erbmusters handelt, mit dem persönliche Merkmale bestimmt werden könnten. Auch eine persönliche Identifikation ist aus diesen Daten nicht möglich. Vielmehr soll die Veränderung der Aktivität der weißen Blutkörperchen durch Ihre Depression sowie die Veränderung der Aktivität im Verlauf der Behandlung beobachtet werden.
- Als letzten Test sollen Sie an einer Geschmacksprobe teilnehmen. Wie Sie möglicherweise festgestellt haben, kann der Geschmackssinn während der Depression verändert sein. Für die Geschmacksprobe wird eine Kochsalzlösung verwendet. Ein Wattebausch wird mit der Lösung getränkt und sie sollen die Salzigkeit und die Angenehmheit einschätzen und auf jeweils einer Skala markieren (Visuelle Analogskala). Dieser Test dauert ca.10 min.

Insgesamt sollte die Erhebung der beschriebenen Parameter nicht länger als eine halbe Stunde nach dem Erwachen dauern. Im Anschuss wird Ihnen ein Fragebogen zu Ihren depressiven Symptomen vorgelegt (QIDS-SR). Sie werden 10-15 min benötigen, um diesen auszufüllen. Im Anschluss danach können Sie frühstücken. Am Vortag werden Sie einen weiteren kurzen Fragebogen (BDI) ausfüllen und es wird ein Interview zu Ihren Symptomen durchgeführt. Dieses Interview beinhaltet die Nutzung von klinischen Fragebögen durch den Untersucher und dauert ca. 45 min.

3. Umgang mit Zufallsbefunden

Die hier durchgeführten Untersuchungen sind keine diagnostischen Untersuchungen, das heißt die Daten werden nicht auf das Vorliegen einer Erkrankung analysiert. Sollten uns trotzdem Auffälligkeiten wie z.B. Herzrhythmusstörungen in der Herzratenvariabilitätsmessung, steile Wellen in der EEG-Ableitung oder weit über die Norm veränderte Laborwerte auffallen, werden Sie darüber von einem Arzt informiert.

4. Datenschutz

Ihre personenbezogenen Daten werden maschinell gespeichert und weiterverarbeitet. Die Weitergabe, Speicherung und Auswertung dieser studienbezogenen Daten erfolgt nach gesetzlichen Bestimmungen ohne Namensnennung mit Codes (z.B. A123) und setzt vor der Teilnahme an der Studie Ihre freiwillige Einwilligung voraus. Einige Laboruntersuchungen werden bei auswärtigen Kooperationspartnern durchgeführt, denen die Zuordnung ihres Namens zu den Untersuchungsmaterialien aber nicht möglich ist.

Wenn weitere Fragen bestehen, werden diese gerne vom Untersuchungsleiter oder dem Projektleiter beantwortet. Bei Ihrer Bereitschaft zur Teilnahme bitten wir Sie, vor der Untersuchung die „Einwilligungserklärung“ vollständig auszufüllen und zu unterschreiben.

PD Dr. med. Carsten Konrad

Einwilligungserklärung

für die Patientinnen und Patienten zur Studie

„Veränderungen des Renin-Angiotensin-Aldosteron-Systems im Verlauf einer stationären Depressionsbehandlung“

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Ich bestätige hiermit, dass ich durch den Untersucher Herrn/Frau ..... mündlich über Wesen, Bedeutung, Risiken und Tragweite der beabsichtigten wissenschaftlichen Untersuchung aufgeklärt wurde und für meine Entscheidung genügend Bedenkzeit hatte.

Ich habe das Informationsblatt gelesen und fühle mich ausreichend informiert und habe verstanden, worum es geht. Der Untersucher hat mir ausreichend Gelegenheit gegeben, Fragen zu stellen, die alle für mich ausreichend beantwortet wurden. Ich hatte genügend Zeit mich zu entscheiden.

Ich habe verstanden, dass bei wissenschaftlichen Studien persönliche Daten und medizinische Befunde erhoben werden. Die Weitergabe, Speicherung und Auswertung dieser studienbezogenen Daten erfolgt nach gesetzlichen Bestimmungen und setzt vor der Teilnahme an der Studie meine freiwillige Einwilligung voraus.

Ich erkläre mich damit einverstanden, dass im Rahmen dieser Studie erhobene Daten/Krankheitsdaten auf Fragebögen und elektronischen Datenträgern pseudonymisiert aufgezeichnet und ohne Namensnennung an der Klinik für Psychiatrie und Psychotherapie der Philipps-Universität Marburg, Rudolf-Bultmann-Straße 8, 35039 Marburg ausgewertet werden oder an eine von dieser beauftragten Stelle zum Zwecke wissenschaftlicher Auswertung weitergegeben und im Rahmen der Studienergebnisse pseudonymisiert publiziert werden.

Ich habe eine Kopie der Patienteninformation und dieser unterschriebenen Einwilligungserklärung erhalten.

Meine Einwilligung, an diesem Forschungsvorhaben als Patient teilzunehmen, erfolgt ganz und gar freiwillig. Ich wurde darauf hingewiesen, dass ich meine Einwilligung jederzeit ohne Angabe von Gründen widerrufen kann, ohne dass mir daraus Nachteile entstehen.

Ich willige hiermit ein, als Patient / Patientin an dem Forschungsvorhaben teilzunehmen.

Name: …….....……..

Geburtsdatum: ……..……

Datum und Uhrzeit: …….……..

Ort: …….....……

Unterschrift des Patienten: …….....

Ich habe den Patienten mündlich über Wesen, Bedeutung, Reichweite und Risiken des Forschungsvorhabens aufgeklärt.

Name: …….....……..

Datum und Uhrzeit: …….……..

Ort: …….....……

Unterschrift des Untersuchers: …….....

6.7. Fragebogen zur Salzpräferenz

Salzpräferenz

Testbogen

Datum & Uhrzeit: _____

Patienten ID: _____

Untersucher: _____

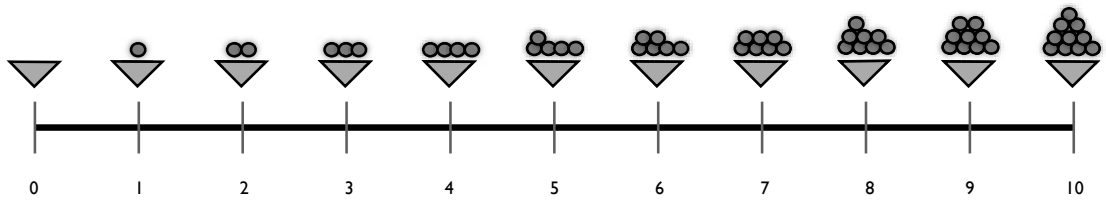
der Studie

„Veränderungen des Renin-Angiotensin-Aldosteron Systems im Verlauf einer stationären Depressionsbehandlung“

1. Mit welcher Salzigkeit würden Sie die Salzlösung auf der visuellen Skala einschätzen?

(Bitte ankreuzen)

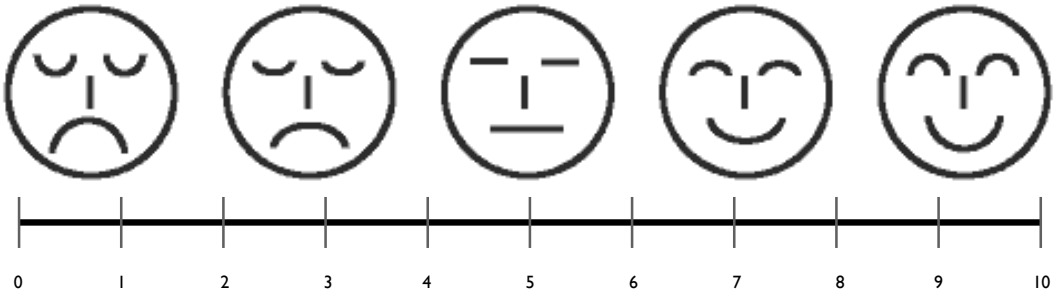
(0 = Kein Salz in der Lösung; 10 = Extrem hoher Salzanteil in der Lösung)



2. Wie haben Sie den Salzgeschmack empfunden?

(Bitte ankreuzen)

(0 = Extrem unangenehm; 10 = Sehr angenehm)



Nur für Personal: Zeitpunkt der Untersuchung: O Baseline O ZP2 O ZP3

7. Formalia

7.1. Verzeichnis der akademischen Lehrer

Meine akademischen Lehrer an der Philipps-Universität Marburg waren (Auflistung in alphabetischer Reihenfolge):

Jürgen Adamkiewicz
Detlef Bartsch
Erika Baum
Klaus Baumann
Stephan Becker
Katja Becker
Siegfried Bien
Stefan Bohlander
Alexander Brehm
Yalcin Cetin
Frank Czubayko
Udo Dannlowski
Jürgen Daut
Richard Dodel
Norbert Donner-Banzoff
Turgay Efe
Volker Ellenrieder
Bilal Farouk El-Zayat
Rita Engenhart-Cabillic
Cornelia Exner
Volker Fendrich
Beate Feuser
Jens Figiel
Susanne Fuchs-Winkelmann
Josef Geks
Christian Görg
Thomas Gress
Ivica Grgic

Kornelia Grundmann
Karl-Heinz Grzeschik
Michael Hertl
Johannes Heverhagen
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Helmut Höffken
Rainer Hofmann
Joachim Hoyer
Walter Hundt
Andreas Jerrentrup
Peter Herbert Kann
Clemens Kill
Tilo Kircher
Klaus-Jochen Klose
Ina Kluge
Michael Knipper
Arne König
Carsten Konrad
Jan Koolmann
Johannes Kruse
Roland Lill
Monika Löffler
Michael Lohoff
Rolf-Felix Maier
Bernhard Maisch
Andrea Maisner
Roland Moll
Ulrich Mueller
Rolf Müller
Harald Murck
Reiner Mutters
Andreas Neubauer
Bernhard Neumüller
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Wolfgang Herrmann Oertel
Dominik Oliver
Egbert Opitz
Axel Pagenstecher
Timothy David Plant
Harald Renz
Gerd Richter
Jorge Riera-Knorrenschild
Manfred Rißе
Volker Roelcke
Steffen Ruchholtz
Helmut Schäfer
Jürgen Schäfer
Stephan Schmidt
Joachim Schneider
Sebastian Schneider
Markus Schofer
Stephan Schulze
Tim Schwarting
Carola Seifert
Jürgen Seitz
Walter Sekundo
Birte Steiniger
Björn Tackenberg
Claus Franz Vogelmeier
Sebastian Vogt
Uwe Wagner
Eberhard Weihe
Jochen Alfred Werner
Rainer Westermann
Christian Wrocklage
Hinnerk Wulf

7.2. Danksagung

Die vorliegende Forschungsarbeit im Bereich der Psychoneuroendokrinologie ist keine Einzelleistung, sondern das Ergebnis einer vielfältigen Zusammenarbeit über mehrere Jahre hinweg zwischen Wissenschaftlern, Klinikern und medizinisch-technischen Assistenten. Ich möchte an dieser Stelle einigen Menschen meinen besonderen Dank aussprechen, die mich während dieser Zeit gelehrt, unterstützt und angespornt haben und in mir ein besonderes Interesse für die Wissenschaft hervorgerufen haben.

An erster Stelle möchte ich meinem Doktorvater Herrn Priv.-Doz. Dr. med. Harald Murck zusammen mit meinem Betreuer Herrn Univ.-Prof. Dr. med. Carsten Konrad danken.

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